

The Ethiopian Calamity

THE attractions of Ethiopia to the tourist are substantial. Daily flights from Europe to Addis Ababa make it an easily accessible country. The last leg of the journey is from Asmara, in Eritrea, to Addis. Beneath this spectacular flight lie the Ethiopian highlands and the extraordinary churches at Lalibela, hewn directly out of the rock. Beneath the flight path and to the east of it, also lie tens of thousands of dead, in regions now gripped by as bad a famine as relief workers have ever encountered.

The region worst affected lies between Makale in Tigre and Dessie in Wallo. In this area there is one rainy period a year, from June until early September; this is the summer monsoon season. As Winstanley pointed out in *Nature* (245, 190; 1973), mean summer rainfall has recently declined from 165 to 100 mm per year for a set of stations across the Southern Sahara to Khartoum. It is this rainfall which makes much of the middle levels (up to 1,500 m) of Ethiopia suitable for cultivation and grazing, and it is its failure that has led to the famine.

In 1972, crops such as millet, maize and sorghum could not be planted. Thus by September 1972 the tragedy had begun. Between then and May 1973, 80% of the cattle were lost and there were smaller losses of camels. In May, the international organisations working in Ethiopia switched their efforts from development to relief and a plea went to the UN World Food Programme for assistance. Aid did not materialise from this source for several months and meanwhile the Ethiopian government, with organisations such as Oxfam and Christian Aid, were trying to cope in a region in which adequate roads were practically non-existent. For several months, however, the world at large was allowed to know nothing as the government kept a very low profile. Meanwhile the Sahelian countries were forthcoming about their similar troubles and received early aid. This cover-up persisted until Ethiopian students in Addis heard of the disaster and approached Mr Jonathan Dimbleby. The result was the powerful film shown on Thames Television two weeks ago. Mr Dimbleby has since broadcast an appeal for financial support for a relief programme which seems destined to continue for at least a year. And still the full extent of the famine is unknown, because Tigre province includes inaccessible villages in which it is feared more may have died.

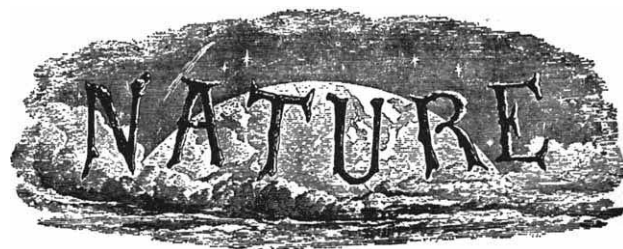
What can be done about this terrible disaster? There is, of course, a continuing need for money to support the concerted actions of the voluntary organisations. There is also a need for technical, particularly medical, personnel to do a short tour of duty in the area. There are, however, many broader issues to face. This catastrophe had been foreseeable since September 1972 and yet nothing was done when there was still time. Further, the severe drought conditions south of the Sahara this year did not seem to alert anyone to the possibility that Ethiopia was having similar problems. One cannot be sure, even now, that all affected regions have been identified—is southern

Sudan free of trouble, for instance? The situation in Ethiopia here and now could have been surmised both by earlier observations in Ethiopia and present observations elsewhere. There is even a large American military communications facility within 150 miles of the region. Yet it needed a television programme to alert the outside world.

No doubt there will be proposals for more technology to improve our understanding of climate, more satellites to keep watch, more equipment in the field, better drought-resistant plants. One cannot but hope that science will provide some future comfort. The central issue, however, has no technological solution; it is a social and political problem and is seen in a most exposed form in Ethiopia. It springs from pride in external relationships and a lack of understanding in internal ones. The ruling race are the Shoans, highlanders centred on Addis, and even a sympathetic observer describes them as "proud, aloof, self-sufficient". It is unlikely that they understand the ways of the herdsmen and farmers in Wallo and Tigre. They certainly know little of the character of the nomadic Danakil people. The present situation finds the central government ill-prepared mentally, however much aid they may distribute. Worse, there has clearly been a great unwillingness to allow external publicity and this can only be attributed to misplaced pride and a fear of loss of tourist income.

It is obvious that as long as governments all over the world are too proud to think beyond national solutions to human problems, and relief agencies are hamstrung by political sensitivities, such tragedies will occur with regularity.

100 Years Ago



THE following awards have been made by the French Geographical Society :—2,000 francs to M. Dournaux-Dupéré, who has just set out for Timbuctoo; this gentleman has also received a similar sum from the Minister of Public Instruction; 2,000 fr. to M. Francis Garnier, to aid him in his explorations along the Blue River in China, and which have Yun-nan and Tibet for their objects; 1,500 fr. to MM. Marche and Compiègne, who have already proceeded a considerable distance along the course of the Ogowe with the design of penetrating as far as the great African lakes, and joining Livingstone.

From *Nature*, 9, 33, November 13, 1873.



The Problem of Compensation for Antenatal Injuries

Dr R. G. Edwards of the University of Cambridge puts forward some personal opinions arising from a discussion at the Institute of Advanced Legal Studies on aspects of fair compensation for injuries sustained before birth.

I WISH to discuss certain points about Working Paper No. 47 of the Law Commission (January 1973) on injuries to unborn children. This proposes statute law for liveborn children suffering antenatal injury to claim damages where negligence of another person can be proven. The concern by lawyers for children with handicaps should be widely welcomed. I have some misgivings, however, about the nature of the proposals, for the correct approach to helping these children should not involve compensation by proof of fault. My doubts arise on several scores:

- The situation created by changes in drug testing methods since thalidomide;
- Difficulty in deciding the causes of many prenatal injuries;
- The possibly complex social consequences arising from legislation, for parents, offspring and doctors;
- The potential unfairness of such legislation to children with birth defects unable to claim compensation under it.

I would also like to explore the possibilities of other approaches to the fair treatment of injuries to foetuses before livebirth.

Causes of birth defects

Many children are born handicapped to varying degrees, and estimates vary between two and six per cent, partly through differing opinions on the nature of the anomalies, and partly because some are manifest long after birth. Some of these handicaps arise genetically, and others through the effects of teratogens during pregnancy. Often there are difficulties in separating the relative contribution of genetic and environmental effects, and interactions between them, and in many cases of birth defects the parents or doctor in attendance will have contributed, accidentally or otherwise.

Teratogens are evidently the main concern of the Commission, and deserve an opening comment. There are practically no guidelines at present in United Kingdom law on compensation for such injury. The unsatisfactory nature of this situation has been highlighted by the recent social debates on compensation for thalidomide children. The funds awarded to these children are obviously a wonderful uplift to them and their parents, and their financial difficulties, at least, should be over. Yet, as well as

the manufacturers, many people and agencies, including the Health Service, were involved in events leading to the thalidomide situation. Responsibilities for drug testing in pregnant animals and for the nature of the tests were diffuse or unrecognised, and fell largely on the manufacturers. This situation has changed since thalidomide, for the governmental Committee on Safety of Medicines now tests new drugs and issues recommendations. In my view, legislation will have to accept this new situation and adapt to it, for a great deal of responsibility for any foetal injury through drugs taken by the mother must now fall on this committee.

My next reservation about the proposed legislation concerns the difficulty in deciding the cause of many congenital defects, for a handicap arising genetically could be mistakenly attributed to exposure to drugs or trauma during pregnancy. There must be many pregnant women carrying a foetus with a genetic defect who are exposed to a potential teratogenic agent, and many misinterpretations in deciding the cause of damage to the child. Indeed, some have already arisen, as judged by some cases in common law. A child was awarded damages for 'imbecile mongolism', judged to arise through injury involving the mother at one month of pregnancy¹. From what we know today about mongolism (trisomy-21), these damages must be unjustified; either a medical misdiagnosis or a miscarriage of justice has occurred, for the chance of a succession of simultaneous chromosomal changes in the cells of a 1-month-old foetus can almost certainly be totally excluded. Similar doubts about the justice of the award were voiced by a dissenting judge in this case. Many of the examples given in the Commission's report appear to be questionable, for club feet and epilepsy evidently have a distinct genetic component²⁻⁴.

Such difficulties seem bound to arise in analyses of the effects of the teratogenic agents quoted in the report; drugs, trauma, X rays and disease. Teratogens usually exert fairly specific effects. One criterion adopted by teratologists is that the type of damage caused must be typical of the agent and of the time during pregnancy at which it was administered. Clear proof of the

effect of teratogenic agents in man will be difficult to obtain; perhaps they could be shown to induce similar anomalies in animal foetuses, but even so the relevance of these observations to man could be questioned.

Trauma is seldom discussed as an agent causing foetal anomalies—foetal death is a more likely consequence. Hardly a case of foetal deformity due to trauma is considered in textbooks, except very recently⁵. The evidence available is shown to be extremely limited indeed; a direct blow to the foetus might inflict damage without death, but clear examples are very hard to prove. Some fairly bizarre examples might arise, such as anoxia following the death of the mother before Caesarean delivery, or accidental damage during surgery, but these hardly seem worth such weight of legislation. Radiation damage is now well recognised, and while mistakes can arise through negligence the best solution to an accidental heavy exposure to X rays might be to abort.

Disease raises very difficult problems. Since it properly includes the effects of German measles and other teratogenic viruses, are there to be penalties for spreading these conditions in a community? The social problems could become even more complex, for foetal handicaps arising from this source would probably be due to parental negligence, so that questions of actions in law by children against their parents would arise, as I shall discuss below. There have been rare cases where the use of contaminated blood⁶ or semen by doctors has led to claims in common law by the deformed child.

Problems in claiming compensation

Much of the proposed legislation could involve claims by children against parents, for example, in the case of congenital syphilis, and once begun such claims could spread to many other situations. Some habits of the mother can impair foetal growth: low birth weights and anomalies in offspring have been reported if the mother smokes or drinks alcohol during pregnancy. There could be grounds for action by children against parents for genetic defects; for example, should a second child have deformities similar to those in an earlier child then the parents might be accused of negligence in the second conception. Claims could even be brought by children for a defective genetic constitution, bastardy or being born half-caste. If the suggestion that these situations could lead to litigation appears to be extreme, let me offer the

defence that these topics have already been discussed elsewhere and some common law cases have actually been heard in court already⁶. The potential upsets to family and social life resulting from numerous claims against parents by children led one court⁷ to decide in favour of the parents even though they were sympathetic to the child's claim, a decision soundly criticised by one legal authority who insisted that such cases should be heard, for if there is a damage then justice must be done⁸. Arguments raised in some related cases led, for example, to justices being compelled to debate the meaning of existence when a child born defective as a result of German measles brought an action against the doctor for wrongful birth⁹.

Further difficult issues may appear with reference to claims arising from agents or acts applied before conception occurred. As far as I am aware, there is no clear evidence, even in animals, to show that specific damage to gametic cells before conception can induce anomaly manifest after birth, with the possible exception of chromosomal imbalance and rare genetic changes. With respect to teratogens, the available evidence shows that eggs and embryos are highly resistant to induced malformation shortly before and after fertilisation. Legislation would therefore be effectively restricted to a few cases. There is also a broader point to be considered concerning the role of doctors and others in helping to establish conception in infertile patients and this is, of course, a field in which I have a direct interest. Many children are now being born after the use of artificial insemination with donor semen, the use of hormones to induce ovulation and other methods of alleviating infertility. Causes for legal action of the liveborn child against the doctor could arise under these conditions, for example, on the choice of the semen donor for AID. A recent report from Germany has implied that children conceived by AID have the right to know the name of their natural father, and doctors refusing to divulge it or creating difficulties in establishing his identity by mixing the semen from several men could be held in law as withholding natural rights from the child¹⁰. Children might be encouraged by legislation to sue the doctor who helped to give life, a possibility more likely than their claiming against parents since the doctor possesses financial resources outside the family. Vital treatment for patients might be withheld because doctors were unsure of their legal position.

These considerations indicate that great care must be taken in framing legislation to cover injury to unborn children. The actual wording of the legislation could lead to further problems. A girl born in 1969 in Vancouver

with defects identical to those of 'thalidomide' children was excluded from many of the benefits of legislation for these children because the real cause of her malformations was unknown (R. B. Lowry, personal communication). I doubt too that the award of damages based on fault to such children is the right or best way to help them in life, with the attendant disadvantages of legal action by a parent or third party sufficiently determined to carry the case through. The legislation would help too few of the children who are afflicted, and probably result in a minority of them obtaining large sums of money while the others depend on state or other benefits. Nor is legislation likely to act as a deterrent, because most injuries will arise accidentally and not with forethought. Granting the right to liveborn children to sue for uterine damage could lead not only to cases of Wrongful Life, but to the more complicated problem of Wrongful Death, in which stillborn foetuses can be the subject of claims, or even have claims brought on their behalf^{10,11}. When these daunting aspects are related to the problems outlined above there would seem to be good cause to search for another method of dealing with foetal injury.

Possible alternatives

An alternative approach can be discerned in existing institutions. The Pearson Commission on Civil Liability and Compensation for Personal Injury has recently been established to consider how compensation should best be payable and financed in respect of death or personal injury arising in various ways, and it will consider antenatal injury. Legislation has already been passed in the United Kingdom to help disabled people (including disabled children) although there are some grounds to believe that the legislation is not being satisfactorily implemented¹². This statute should be fully implemented to provide generous rehabilitation facilities by the state to all children with congenital injuries, whatever the nature of their origin, and so increase the effectiveness of the measures now available. The statutory provisions should be sufficiently generous to take care at least of the medical and social urgencies associated with physical handicap, education, vocational training and employment, and protect the parents as far as possible from suffering and financial loss; some countries have already taken such steps¹³. An important aspect in financing could be to establish a system of "liability without fault" for antenatal injuries, such as the Pearson Commission is considering. Insurance or other systems would be needed to raise funds.

A further lead can be gleaned from the work of the Committee on Safety of Medicines which has statutory authority to oversee and control new

developments in its field. This or another committee could be given wider authority to extend current schemes, mostly local, to monitor the incidence and type of birth defects (chronically sick and disabled persons, including newborns, are now registered by local authorities under the Chronically Sick and Disabled Persons Act (1970)¹⁴). It could also take some of the responsibility for advising and helping doctors and others in making decisions about the value of new methods, and undertake some responsibility to oversee the rights of foetuses. A danger of such a committee might be delay in the application of new methods, unless such developments were excluded from the scope of its deliberations. Within this framework, some organisation might be created to oversee conditions in this field, if necessary having the right to bring actions for negligence against offending parties, and providing legal and medical help where needed. In a different context, compensation for injuries arising to adults through criminal or other action can be awarded by a board, and guardians for unborn children have been legally appointed in the past⁶. I do not know if such compensation could be conferred within our legal situation, although funds can be provided in various ways, for instance, by Royal Prerogative exercised by the Home Secretary in the case of injuries through criminal action.

Ultimately, expenditure of public funds would need legislation, and suitable steps would be required to provide any such committee with appropriate authority. In the meantime, exploratory steps could be taken, or some other systems proposed, in order to avoid many of the pitfalls accompanying legal questions concerning parents, children and other parties, and yet give maximum support to those needing it most—the children afflicted at birth.

¹ Sinkler v. Neale, 401, Pa. 267, 164 A 2d 93 (1960).

² Carter, C., & Morton, E., in *Congenital Malformations* (Excerpta Medica, 1969).

³ Kalter, H., *Teratology of the Central Nervous System* (Chicago, 1968).

⁴ Wilson, J. G., *Environment and Birth Defects* (Academic Press, New York and London, in the press).

⁵ Bundesgericht 20 XII 1952, *Juristenzeitung*, 307 (1953).

⁶ Tedeschi, G., *Israel Law Review*, 1, 513 (1966).

⁷ Zepeda v. Zepeda, 4, 111 App. 2d 240, 190 NE 2d 849.

⁸ Gleitman v. Cosgrove, 49 NJ 22, 227 A 2d 689 (1967).

⁹ *Frankfurter Allgemeine Zeitung*, January 3, 1973.

¹⁰ Fowler v. Woodward, 138 S.E. 2d 42 (S. Car., 1964).

¹¹ Quinn, A. J., and Griffin, J. A., *The Jurist*, 31, 577 (1971).

¹² Morris, A., *The Times*, June 19, 1973.

¹³ Lind, J., in *Congenital Malformations* (Excerpta Medica, 1969).

¹⁴ Morris, A., & Butler, A., *No Feet to Drag* (Sidgwick & Jackson, London, 1972).

NEWS

Canadian Societies seek More Involvement

from a Correspondent

THE Royal Society of Canada, together with others among Canada's 119 engineering, scientific and technological societies, has been absorbed recently in a searching re-evaluation of its functions and its proper role. One sign of the society's attempts to move with the times was its sponsorship last month of a symposium on energy resources. Some observers in Ottawa are wondering whether the symposium reinforced, rather than changed, the society's public image as an ultra-conservative club for senior scientists, as the meeting was almost entirely devoid of either controversy or confrontation. It is worth recapitulating some of the background to this attempt at increased involvement.

Last December the Science Council published a study* which recommended a more active role for scientific societies in dealing with the implications of science and technology for society. It was also proposed that the federal government should establish a 'Canadian House of Science, Engineering and Technology' to provide the societies with offices and meeting space for their secretariats.

Written by Professor A. S. West, an entomologist from Queen's University, the study also noted the following difficulties facing most Canadian scientific societies:

- They are small and compete with larger counterparts in the United States;
- They have low fees and small incomes, yet must cope with the expense of bilingualism;
- Their young scientists are too little involved and often have a poorly developed social conscience.

Professor West did his study under contract from the Science Council of Canada through an organisation known as SCITEC (the Association of the Scientific, Engineering and Technological Community of Canada). SCITEC was formed in 1970 in response to the complaint of members of the Senate special committee on science policy (the Lamontagne Committee, see *Nature*, **245**, 406; 1973) that they could not find a single voice to speak for all of Canadian science. It has since abandoned any pretence of fulfilling such an oracular function, but is now the principal contender, together with the Royal Society, for leadership among Canadian scientific societies.

The two societies are quite different

in purpose and structure. The Royal Society is the Canadian equivalent of an academy of science, but SCITEC is somewhat analogous to the British or American Associations for the Advancement of Science. Both groups claim, however, some similar functions. One of these is to increase public understanding of scientific issues; SCITEC itself had already held a symposium on the social and economic implications of the energy situation.

The report of the Lamontagne Committee proposed that the two organisations should not compete but cooperate, by defining and dividing their functions. "The Royal Society," it said, "with its reservoir of scholarship represented by its fellows and scientific panels could concentrate its national activities on the use of science for policy—on scientific and technological studies dealing with Canadian issues. SCITEC, as an overall spokesman for the engineering, scientific and technological societies, could specialise on policies for science and technology—on broad issues concerning scientists, engineers, and technologists in the exercise of their professions."

The Royal Society's latest symposium, however, seems to some to cast doubt on the claim of its president of last year, Professor Tuzo Wilson, that the organisation is being revitalised. For although the subject matter (energy) was highly topical, the approach and the choice of

speakers and subjects seemed conventional and the papers added nothing new to an already much-debated problem. What little discussion was allowed, was conducted by means of written questions; a practice that brought a formal complaint from some of the younger members of the audience.

There were some good things. The society took unusual steps to publicise the venture and had a good attendance as a result (some 300). The review papers provided a mass of information. And three of the thirty-five speakers departed from the predominant theme—how to increase the energy supply—by proposing that perhaps what is needed is a whole new policy of moderation of life styles to slow down the rate of increase in the use of energy. One of the three was former chairman of the Economic Council of Canada, John J. Deutsch.

In his report, Professor West spoke of the need of a major overall co-ordinating organisation to increase the involvement of Canadian scientific societies, and he quoted an unnamed scientist as saying it must be "of such stature and articulation that the public will accept its pronouncements on the same plane at least as those of the Science Council."

The impression remains that, in spite of recent attempts, neither society has yet succeeded in fulfilling that role.

MEDICAL JURISPRUDENCE

Transplants and Murder

by our Washington Correspondent

A MURDER trial which is to open in California on December 18 will be the venue for a legal battle over the precise definition of death, and the outcome will have significant implications for transplant surgery. At the centre of the trial is Andrew D. Lyons, who is accused of shooting Samuel Moore, a 29-year-old California man, in the head on September 10, 1973. On September 12 Mr Moore's brain showed no signs of electrical activity, he was unable to breathe, and did not respond to stimulation. He was then pronounced "neurologically dead", and his heart was used in a transplant operation performed by Dr Norman Shumway, a Stanford heart surgeon who has performed some 65 transplants; 23 of his patients are still surviving.

Californian law allows transplants to be performed provided that the donor has been pronounced dead, but no pre-

cise legal definition of death is given. In this case, according to the *New York Times*, Moore's wife and mother had given permission for the transplant, Moore was pronounced dead on the grounds of a lack of cortical activity, and his heart was removed while still beating. Clearly, the concept of brain death is going to play a large part in the trial. There is, however, one close parallel case in American legal history. Last year, a team of surgeons from the Medical College of Virginia was acquitted of a charge of killing a 56-year-old black man to use his heart in a transplant operation. In that case the donor suffered brain damage as the result of a fall, and he was pronounced dead when an electroencephalogram showed no signs of electrical activity in the brain. His heart was subsequently removed, while still beating, and used in the first heart transplant carried out in Virginia. The jury in the trial had little difficulty in deciding that the donor was legally dead when his heart was removed, and the doctors were acquitted.

* *National Engineering, Scientific and Technological Societies of Canada*, A. S. West, Science Council Special Study No. 25.

Short Notes

Le Ballon Rouge

THE first part of a new Franco-Soviet drift balloon project, to investigate high altitude phenomena at Arctic latitudes, will soon begin with the launching of a balloon from a test site in Sweden. The experiment, called "grebenka" (comb), is intended to monitor charged particles and associated phenomena during the aurora borealis, and will consist of two series of balloon flights, the first in winter 1973-74 and the second a year later. The flights will be at an estimated altitude of 35 to 45 km, and due to the strong westerly prevailing winds are expected to proceed eastwards from Sweden over the northern islands of the Ural ridge for a distance of some 2,000 km.

According to *Pravda* (October 31, 1973) the balloon is to be tracked throughout by radar and the data gathered are to be transmitted to the ground station and recorded there for subsequent analysis—a somewhat surprising procedure, since it is explicitly stated that one of the objects of grebenka is to monitor those conditions in which radio communications break down.

Skylab Launch Date

THE launch of the third and final crew of Skylab has now been scheduled for November 10. This is earlier than some astronomers had hoped, and it seems that Skylab will, after all, be unmanned for part of the period in January when Comet Kohoutek is expected to be at its most spectacular. But according to NASA "consumables will be closely husbanded to keep open an option to extend the final mission to about 70 days". Kohoutek will reach perihelion on December 28, and a "standard" 56 day mission would terminate on January 6. But if the mission does run until January 20, 1974, or thereabouts, there would be opportunities for studying ice and snow distributions in the northern hemisphere, and the start of the growing season, as well as making further observations of Kohoutek. The two earlier Skylab missions lasted for 28 and 59 days.

Russian Nuclear Activity

THREE underground nuclear explosions were fired on October 26 and 27 in the Soviet Union in the most vigorous burst of activity ever recorded. One shot was in the normal testing ground near Semipalatinsk, and a second was on the Arctic island of Novaya Zemlya. This latter was the largest ever fired underground in the Soviet Union and was comparable in yield to the Cannikin blast in the Aleutians in 1971. Several aftershocks were recorded from the event. The largest had a magnitude of 4.6. This is the first occasion on which

aftershocks of Soviet tests have been reported. The third shot was in the Urals region and was in all probability for peaceful purposes. Soviet Plowshare activity in the last two years has been at the high level of between ten and twenty shots each year.

Winning Sargassum War

ATTEMPTS to clear the Japanese seaweed *Sargassum muticum* from the Isle of Wight and Portsmouth harbour are proving successful. The weed is being collected fortnightly and the majority of fronds are only 20 to 30 centimetres long—the length to which they grow in about a fortnight.

As yet a committee formed by local authorities with representation from the Nature Conservancy the Ministry of Agriculture, Fisheries and Food, the local river authorities and the Natural Environment Research Council (NERC) has still to meet to decide if money is to be allocated to help with the eradication.

To date the £500 spent on clearing the weed has all come from Portsmouth Polytechnic, the Nature Conservancy and a local trust, with studies of the seaweed's behaviour being financed by NERC. Local authorities have still to make a contribution.

Dr Gareth Jones, of Portsmouth Polytechnic, who has been organising the eradication programme says that "at the moment, there is no real threat as we are digging the seaweed up, but come next spring there very well could be a problem. It is under control now; it does not necessarily mean that it will be by then".

To keep the weed—which grows at the alarming rate of 1 to 2 centimetres a day and which could create a hazard for shipping, cover oyster beds and foul beaches—under control, a full-time appointment is needed. To ensure that the weed does not become established in Britain the young plants that are springing up will have to be removed regularly for the next 2 or 3 years.

Centrifuge Setback

THE United States gas centrifuge programme for enriching uranium received a severe setback on October 20, when a model centrifuge was destroyed in an accident at the Oak Ridge plant in Tennessee. The accident, which was made public by the Atomic Energy Commission last week, occurred when an advanced model centrifuge failed at high speed, damaging several other units and releasing 4 ounces of uranium hexafluoride. By good chance, the accident occurred on a Saturday, when few people were in the building, and nobody was hurt. What effect the accident will have on the programme remains to be seen, and the AEC is not yet prepared to give many details away. An investigation is under way, however, and its

findings will determine the extent of the adjustments, if any, that will have to be made in the design and operation of its other centrifuges.

USGS Gets the Lot

THE United States Geological Survey has inherited all the seismological, geomagnetic and earthquake research programmes from the National Oceanic and Atmospheric Administration (NOAA). Prime object of the transfer is to save money by consolidating the Geological Survey's extensive activities in Earth sciences with those of NOAA, and to cut down on the duplication of effort which has been going on, particularly along the San Andreas Fault. The first stage of the transfer took place in June, when NOAA's Earthquake Mechanisms Laboratory in San Francisco was merged with the Geological Survey's National Center for Earthquake Research in Menlo Park. The second stage, announced last week, completes the transfer of NOAA's entire programme, including management of the Worldwide Standard Seismograph Network, the National Earthquake Information Service and all the seismological observatories except for three needed for the tsunami warning system. As far as the first stage of the transfer is concerned, it seems that difficulties have arisen because the two laboratories were doing parallel work, and redundancies are expected.

Comet Kohoutek Limited

Two London businessmen have come up with the bright idea of registering the name 'Comet Kohoutek' for business purposes. Operating as Comet Kohoutek Limited, they are at present negotiating with "a number of national advertisers" in Britain intent on using the comet to promote such items as toys and fireworks. A spokesman for the company said this week that one national newspaper has already come to an arrangement with Comet Kohoutek Ltd about use of the name, but he could not give any details before the promotion gets under way. Members of the scientific and journalistic communities will no doubt be relieved to learn that they can use the name Comet Kohoutek in describing the comet without fear of prosecution; but anyone who is based in Britain and wants to market, say, a new drink called 'Kohoutek—the fizz with a wizz' will have to pay for the privilege. Comet Kohoutek Ltd operates from 11 Rammore Court, Catherine Road, Surbiton, Surrey KT6 4HE.

No Comment

"It will add considerably to our inadequate knowledge in the ill-documented field of . . ."—Book advertisement in a recent issue of *Nature*.

NEWS AND VIEWS

Hot Spots on X-ray Sources

THE study of X-ray sources is one of the great success stories of present-day astrophysics. For objects like Cen X-3 or Her X-1 it is possible to build up a model which is based, almost entirely, on qualitative arguments. Thus the X-ray observations of these sources show short period fluctuations in brightness, on a time scale of seconds. No simple astronomical object can oscillate or rotate as fast as this unless it is considerably denser than a white dwarf. The next densest type of object is a neutron star, and so the first qualitative conclusion is that the X-ray emission comes from neutron stars (at least).

Superposed on the short period fluctuations there are longer period variations, very reminiscent of those seen in eclipsing binaries. Optical astronomers have long been very fond of such close binaries, for their detailed observation readily permits the determination of interesting physical parameters, such as the masses and the radii of the partners, and their distance apart. More than that, it has been realized in recent years that the close proximity of one star to another may lead to great perturbations in the behaviour of one or both members of the pair. The most important of these effects is a strong tidal distortion, which allows mass to stream out of one star and into the other.

These ideas have a counterpart in the case of the X-ray sources. The generally accepted model has it that the binary consists of a rather ill-matched pair of stars. The primary star is large and diffuse; once in each period it eclipses the secondary, which is very compact, and which also emits the X rays. Because the secondary emits so much energy (in the form of X rays) it will cause a considerable modification in the properties of the atmosphere of the primary. It is quite possible that most of the radiation from the primary is really derived from energy generated on the X-ray source. It is equally likely that the uneven heating caused by the X-ray source will lead to the loss of mass from the outer layers of the primary.

Here is a crucial point in the whole description, for it is this very gas, which the X-ray source extracts from the primary, that finally falls on the neutron star and provides the energy for the X-ray emission. The energetics of the process can be quite satisfactorily understood. It requires of the order 10^{15} erg of energy to pull one gram of matter off the surface of the primary. But about 10^{20} erg can be recovered for each gram that falls on the neutron star. Once the pump is primed it should go on running quite satisfactorily even if only a small fraction of the gas finds its way to the neutron star, and even if most of the X-ray energy escapes into space.

Now how does this gas get down onto the surface of the neutron star? From the theory of pulsars one knows that neutron stars often have strong magnetic fields. In the case of the X-ray source the infalling material, which will of course be highly ionized, must be guided down to the surface along the lines of force of the magnetic field. The outermost lines of force in the field are those which originate at the magnetic poles.

So the gas falls down a pair of 'funnels', each of which terminates at a magnetic pole. This is where the potential energy released in the fall is turned into X rays. As the X-ray source turns the hot spot(s) can intermittently be seen. Hence the pulsation of the source.

This is indeed a persuasive picture, but it is based on more hand-waving than even an astrophysicist is likely to be used to. Nobody can yet describe exactly how the uneven heating by the X-ray source affects the thermal balance of the atmosphere of the primary, nor how much mass loss results from it. Nobody can yet say how the circumstellar plasma will interact with the low frequency radiation from the rotating neutron star. Finally, nobody can yet give a hydromagnetic description of the process by which the neutron star captures the plasma. But at least a start has been made on a description of the last stages of the infall.

In an article in last Monday's issue of *Nature Physical Science* (246, 1; 1973) Davidson discusses the mechanics of the plasma flow in the region just above the magnetic poles. Here the infalling gas is constrained to move along the lines of force, which are almost rigid because the magnetic field is so strong. As it comes close to the surface, the gas is decelerated by radiation pressure. But because it is slowed down, it must lose energy, which it gives up to the radiation field, thereby adding to the radiation pressure. It turns out that the radiation cannot escape upwards against the gas flow. Instead it leaks away through the sides of the funnel.

Comparison shows that this relatively simple sequence of ideas can be brought into good agreement with observation. A set of quite homely parameters describes the mound of gas where the X rays are produced. It is about as high as the Post Office Tower, covers an area somewhat smaller than Hyde Park, and the mass per unit area in the mound is about the same as the mass per unit area in the Earth's atmosphere.

It seems out of the question that these predictions will ever be verified by direct observation. On the other hand, it is unlikely that anyone will ever seriously challenge these conclusions, except perhaps in some minor way.

F. D. K.

Lymphocyte Trapping

CELLULAR immunologists have been so enthusiastic in their pursuit of knowledge that they have described many phenomena of which, at present, the biological significance is uncertain. Allogeneic inhibition, for example, which related to various situations in which foreignness of environment was apparently inhibitory to a wide variety of cell populations, was popular a few years ago. Nobody knew whether it was significant in relation to the growth of a tumour, or a kidney allograft, but it was, and presumably still is, an interesting phenomenon. Perhaps the discovery of allophenic mice in which genetically very disparate cells seem to exist in cheek and jowl in perfect

harmony presaged the decline from favour of the notion of allogeneic inhibition. Lymphocyte trapping, the subject of a communication by Frost and Lance on page 101 of this issue of *Nature*, is similarly a phenomenon widely thought about but of which the biological significance is unclear.

In earlier papers Lance and his colleagues have described how, when mice are injected with ^{51}Cr -labelled syngeneic lymphocytes during an immune response, the label appears preferentially in the responding lymphoid organ. Other immunologists have shown a small drop in lymphocyte output from lymphoid organs in the early stages following antigenic stimulus, and it seemed to Lance and his colleagues reasonable to suppose that during an immune response there were alterations in the flow of lymphocytes through the responding organ such that more cells were, in the Lance terminology, trapped. It has been found that although trapping can occur during an immune response, the mechanism is more or less independent of the immunological status of the organism in which trapping is demonstrated.

In their new paper Frost and Lance describe how trapping is much reduced in both the spleen and lymph nodes during a response to antigen in mice which also carry certain transplanted ascites tumours. Ascitic fluid injected into non-tumour bearing recipients had a similar depressing effect on the trapping system. As it was shown in other experiments that the same tumours growing in a solid condition failed to alter the capacity to trap—which indeed was augmented in lymph nodes draining the site of implantation of the tumour—the authors could not argue that malignancy *per se* was having an immunosuppressive effect. Nevertheless, they postulate that part of the immunosuppression sometimes found in advanced cases of malignancy could be due to failure of trapping.

It will be of considerable interest to find out whether the mice with ascitic tumours had similar factors in their serum to those found in shock plasma which can inhibit some of the activities of the reticuloendothelial system. It could be that trapping is a manifestation of stimulation of the macrophages and that in various 'stress' situations trapping is reduced because of partial failure of excitation of macrophages.

From a Correspondent

EPIDEMIOLOGY

Sexual Transmission of Hepatitis B

from our Medical Virology Correspondent

THE epidemiological concepts of hepatitis B infection have recently undergone a significant change. The demonstration that the hepatitis B agent is infective by mouth and the finding that the infection is endemic in closed institutions, the prevalence of infection in adults in urban communities, the carrier rate and age distribution of hepatitis B antigen in different geographical regions and the relatively high incidence in poor socio-economic environments, have altered the epidemiological dogma that hepatitis B is spread exclusively by blood and blood products through the direct parenteral or percutaneous route. Some evidence is now also available for the transmission of hepatitis B by intimate personal contact and possibly by the venereal route.

In 1971, Hersh and colleagues (*New Engl. J. Med.*, **285**, 1363) presented the case history of six patients with hepatitis B infection. Although the precise mode of transmission from a man to his close female companion or companions was not clearly defined, a non-percu-

taneous route was strongly implicated. The first patient developed hepatitis 3 months after blood transfusion, hepatitis B antigen persisted in his blood and 3 months later his wife developed antigen-positive infection. The second patient was a drug addict who contracted acute hepatitis B; his wife, who was not an addict, developed hepatitis without jaundice 4 months later and the antigen was detected transiently in her serum. The third patient suffered from hepatitis and his girl friend developed a similar illness 3 months later. The fourth patient was a technician working in a renal dialysis unit and hepatitis B antigen was detected in his serum during routine examination. Three of his intimate female contacts developed acute hepatitis B infection. Another patient developed acute hepatitis B; her husband had chronic hepatitis and the antigen was repeatedly found in his serum. The sixth patient in this series was a drug addict, who died of acute fulminant hepatitis. His girl friend, who did not take drugs, suffered from acute hepatitis 2 months later.

CEA-like Substance on Erythrocyte Membranes

IN 1965 Gold and Freedman described an antigen which was thought to be specific for human tumours and foetal tissues of endodermal origin. Called carcinoembryonic antigen (CEA), it was a finding of potentially great importance. But it was not until about four years later, when the first CEA radioimmunoassays were reported, that the CEA story really began to interest the scientific community. Such methods suddenly gave rise to the possibility of being able to perform early screenings for the deadly colonic carcinomas, at a time when the disease would not yet be clinically apparent. Sadly, more recent experience has shown that this hope was over-optimistic; for colonic CEA, or substances cross-reactive with it, has been detected by various methods to occur in the urine, plasma, or faeces of patients with both neoplastic and non-neoplastic (usually inflammatory or regenerative) disorders, and even, albeit in much smaller amounts, in apparently healthy subjects.

It is conceivable that the occurrence of CEA (a glycoprotein molecule), in non-neoplastic conditions, could be, in part, due to the release of glycoprotein moieties from the various types of cells which have cross-reacting antigenic specificities with known colonic carcinoma CEA. This possibility is strengthened by results of Nery, Bullman and Barsoum described in *Nature New*

Biology next Wednesday (November 14).

Noting that the levels of CEA in the plasma of fresh blood specimens may increase on storage of the whole specimen, Nery *et al.* set about to see if CEA-like cellular components are gradually released into the plasma by red cells. Using isolated red cell membranes they indeed detected CEA by radioimmunoassay; furthermore, red cells from cancerous and non-cancerous patients as well as healthy control donors were positive and all gave similar mean values. Further analysis of the CEA-like substances demonstrated that they have glycoprotein and glycolipid structures, partially cross-react immunologically (as defined by double diffusion in agarose) with known colonic carcinoma CEA, and have molecular weight characteristics similar to colonic carcinoma CEA. The CEA activity was assessed to be probably more than a single structural entity.

It would seem, as Nery *et al.* cogently point out, that what is known as 'immunologically defined CEA' may in fact represent a family of antigenically related molecules, possessing subtle structural differences, perhaps resulting from impaired cellular metabolism. For those who are concerned with assay of CEA, or indeed of any purified tissue antigen, the implications are self-evident.

Mosley (*Can. med. Ass. J.*, **106**, 427; 1972) reported further data which implicated intimate personal contact. During a study of the secondary attack rates of hepatitis among household contacts of patients treated by maintenance haemodialysis the incidence of the infection among children and non-spouse adults was 1.4%, whereas the rate in marital partners was 20%.

Three more recent studies in London tend to substantiate the possibility that sexual transmission of hepatitis B infection may be a real entity. Jeffries and colleagues (*Brit. med. J.*, **2**, 455; 1973) examined serum specimens from 1,650 patients attending a venereal disease clinic in London. Hepatitis B antigen was detected in the serum of 2 out of 553 (0.4%) female patients; 1 out of 520 (0.2%) European male heterosexual patients; and in 11 out of 282 (3.9%) European male homosexuals. The possibility of venereal transmission of hepatitis B infection in homosexuals had been suggested previously. Many of the homosexual patients in this survey attended the clinic repeatedly with genital and skin sores and transmission of hepatitis may have occurred through these lesions.

In another survey conducted by Fulford and colleagues (*Lancet*, *i*, 1470; 1973), sera from 974 patients attending another clinic for sexually transmitted diseases were tested for hepatitis B antigen and antibody. The antigen was detected in the serum of 1.7% of the clinic patients and antibody in 4.8%, whereas in the new blood donor population the overall prevalence was 0.2% and 0.4% respectively (bearing in mind, of course, that these two populations are not strictly comparable). Statistical analysis revealed significant differences for sex and marital status, with both the antigen and antibody-positive groups containing a higher proportion of single men. Highly significant correlations were found for the hepatitis B antibody group with a history of both syphilis and gonorrhoea. Patients with antigen had a significantly higher frequency of gonorrhoea. It was again noted that the group with hepatitis B antibody (which constitutes evidence of past infection with hepatitis B virus) contained a much greater proportion of homosexuals and also promiscuous patients of British origin. In yet another investigation on the mode of spread of acute hepatitis B (Heathcote and Sherlock, *Lancet*, *i*, 1468; 1973) sexual contact or close domestic contact seemed to be the most likely source of infection in 40% of 67 patients studied.

It seems, therefore, that the concept of hepatitis B as an important sexually transmitted infection merits careful consideration not only because it represents a new epidemiological entity but also because prevention may be feasible.

RIBOSOMAL RNA

Control of Synthesis

from a Correspondent

ALTHOUGH the multiple ribosomal RNA genes (rDNA) of *Xenopus laevis* are probably the best characterised of any eukaryotic organism, very little is known about the control of rRNA synthesis. The genes are linked at one locus which is very closely associated with the nucleolus organiser region, since a deletion, which obeys simple Mendelian rules, eliminates both rDNA and nucleolus organiser together. Each gene consists of two parts; the transcription unit which produces ribosomal precursor RNA containing 18, 28S and excess transcribed spacer sequences, and a spacer unit which is not transcribed. Another species of *Xenopus*, *X. mulleri*, also has multiple rRNA genes and a detailed comparison (Brown, Wensink and Jordan, *J. molec. Biol.*, **63**, 57; 1972) between the rDNA of *mulleri* and *laevis* has revealed several interesting things.

Briefly the two sorts of rDNA have different base compositions which is reflected in the slightly greater buoyant density of *laevis* rDNA. Even though it is impossible to distinguish between *laevis* and *mulleri* 18 and 28S rRNA on the basis of size, base composition, or fidelity of nucleic acid hybridisation, the two precursor rRNAs may be distinguished by nucleic acid hybridisation in the presence of excess 18 and 28S rRNA. The species difference is thus due to altered base sequences within the transcribed and non-transcribed spacer regions.

In the latest issue of the *Journal of Molecular Biology* (**80**, 217; 1973) Honjo and Reeder describe the synthesis of rRNA in hybrid frogs produced by crossing *Xenopus laevis* and *mulleri*. Using the notation I^+/I^+ and m^+/m^+ to describe the homozygous wild type ribosomal genotype of *laevis* and *mulleri* respectively and I^-/I^+ to describe the *laevis* heterozygote ribosomal genotype (a similar m^-/m^+ genotype has not been described in *mulleri*), it is possible to write the genotypes of the four possible hybrid animals as follows: I^+/m^+ , m^+/I^+ , I^-/m^+ , and m^+/I^- , given that the maternal contribution is written first.

What Honjo and Reeder have done is to measure the relative transcription of *laevis* and *mulleri* rDNA in these four hybrid types at different stages of development as well as in adult animals. Since it is impossible to distinguish 18 and 28S rRNA from the two species, they had to develop a technique for measuring relative amounts of *laevis* and *mulleri* rRNA based on detection of the non-conserved regions of the precursor rRNA. This was done by hybridisation of a mixture of authentic ^3H -labelled *laevis* precursor rRNA with ^{32}P -labelled precursor rRNA from the

hybrid animal to purified rDNA from both *laevis* and *mulleri* in the presence of excess unlabelled 18 and 28S rRNA. The results are expressed as the ratio (L/M) of $^3\text{H}/^{32}\text{P}$ bound to *laevis* rDNA (L) to $^3\text{H}/^{32}\text{P}$ bound to *mulleri* rDNA (M). They constructed a standard curve of the ratio L/M against the proportion of *mulleri* rRNA in a known mixture of ^{32}P -labelled *mulleri* and *laevis* rRNA and used this for assaying unknown mixtures.

The results of their experiments are briefly as follows: when a hybrid receives *laevis* rDNA (that is, genotypes I^+/m^+ and m^+/I^+), *mulleri* rDNA is repressed; but when a hybrid receives no *laevis* rDNA (that is, genotypes I^-/m^+ and m^+/I^-), *mulleri* rDNA is repressed only if the maternal contribution is *laevis* (I^-/m^+) and then repression is only transitory since by 100 h of development synthesis of *mulleri* rRNA is the same as in the m^+/I^- hybrid which shows no repression of the *mulleri* rDNA.

Honjo and Reeder interpret these results as indicating two pathways for the repression of *mulleri* rDNA. One, due to the presence of *laevis* rDNA, is permanent and not maternally inherited, and the other, due to the presence of *laevis* egg cytoplasm, is maternally inherited and transitory. Clearly these observations may represent two points on the same repression pathway; the cytoplasmic maternally inherited repressor may be identical with the diffusible intermediate operating in repression by *laevis* rDNA, though as they point out the mechanism of repression by *laevis* rDNA may be due to its higher affinity for some molecule in short supply and not to its coding directly for a repressor molecule.

Regardless of the details of the repression mechanism, it seems fair to say that it has something to do with the spacer regions of rDNA since there are apparently no differences between the 18 and 28S rRNA sequences of the two species themselves, and the spacer regions are known to have diverged. This suggestion relies on the assumption that the I^- deletion of rDNA and nucleolar organiser removes no other genetic component which might act as the source of repressor. In this connection it would be interesting to know whether and to what extent the partial nucleolar mutants (Miller and Gurdon, *Nature*, **227**, 1108; 1970; Knowland and Miller, *J. molec. Biol.*, **53**, 321; 1970) of *laevis* will repress *mulleri* rDNA.

Laevis/mulleri hybrids have also previously been used to distinguish between 'chromosome copy' and 'episome' models for rDNA amplification during oogenesis (Brown and Blackler, *J. molec. Biol.*, **63**, 75; 1972). Brown and Blackler found that, regardless of whether *laevis* was the male or female parent, hybrid female animals always amplify

the *laevis* rDNA even though they contain both *laevis* and *mulleri* rDNA complements, which is consistent with the 'chromosome copy' mechanism but not with the 'episome' model since the latter demands maternal inheritance. It is not known at present whether the mechanism for repression of *mulleri* rRNA synthesis has anything to do with the selective amplification of *laevis* rDNA, although the mechanism of rDNA amplification proposed by Crippa and Tocchini-Valentini (*Proc. natn. Acad. Sci. U.S.A.*, **68**, 2769; 1971) based on RNA-dependent DNA synthesis would specifically predict amplification of only *laevis* rDNA if *mulleri* rRNA synthesis is repressed.

WILDLIFE

Death on the Road

from a Correspondent

THE question of what motivates a domestic fowl to traverse a highway has occupied the mind of man ever since roads were invented and chickens were domesticated. This problem was not solved at a symposium entitled "Motorways and the Biologist" which was held at the North East London Polytechnic on October 25, but similar questions were raised; such as the reason why deer and badgers make suicidal excursions across trunk roads and motorways and how they can be discouraged.

D. Venner (Forestry Commission) described attempts to discourage deer from crossing roads in the Forest of Dean by mounting 3-inch square mirrors on posts at the side of the roads which reflect motorcar headlights into surrounding woodland and give animals advance warning of approaching vehicles. The highly predictable nature of deer in both the time and location of crossing roads can be used to advantage in planning the siting of such mirrors. Unfortunately, the data on deer mortality before and after the erection of mirrors are ambiguous and adequately controlled experimentation is needed before this system can be evaluated.

Badgers are also creatures of habit and they often insist on using their well-worn trails between sett and foraging ground even when a motorway is constructed across them. M. Clark and E. P. Ratcliffe described experiences in Hertfordshire and Cheshire respectively in which elaborate attempts have been made to accommodate the inflexible attitudes of local badgers disturbed by motorway construction. In certain situations, where badger trails are well known, special culverts have been incorporated into the design of new motorways, together with badger-proof fencing which channels the animals into the tunnels. Stream culverts can easily

be modified for badger use, but it costs £1,200 to construct a culvert 1 m in diameter especially for badgers. One must admire the motivation behind such expenditure, but so far only circumstantial evidence exists that badgers have demonstrated their appreciation by using these tunnels. It is possible that in the long term a great deal of money could be saved if research into such problems were commissioned before such expenditure was incurred.

The other major topic considered at this meeting was the value of Britain's motorway verges for wildlife conservation. J. M. Way (Nature Conservancy, Monk's Wood) has conducted a survey of the plant life of the M1 motorway between Hendon and Leeds and has found more than 380 plant species on its verges. It is possible to devise management procedures in which the demands of the highway engineer can be reconciled with the desires of the botanist. Some of the most interesting plant communities of verges develop in situations where rocks such as chalk and limestone, which may be exposed by excavation, have not been covered by topsoil after construction. Perhaps arrangements could be made to leave such areas bare. Herbicide treatment is obviously not conducive to a varied flora, nor is it necessarily in the economic interest of those managing a verge. Experiments on cutting frequency reveal that just two mowings, one in May and a second in June, can be effective in maintaining vegetation below 30 inches in height (the recommended level), thus avoiding any herbicide treatment.

The conservation of roadside flora may have some economic as well as amenity value. J. Free (Rothamsted Experimental Station) in a survey of the M1 near St Albans found sixty-seven insect species, of which hoverflies constituted the most important group, carrying pollen on their bodies from a total of thirty-nine plant species. Verges with a high floral diversity may thus act as reservoirs for such beneficial insects.

A further consideration in the conservation of verge flora and fauna is pollution. P. Williamson (Portsmouth Polytechnic) described a survey of lead pollution on the verge of A1 (M) at Catterick. Traffic levels during the survey period were 34,000 vehicles per day. Lead, presumably originating from exhaust fumes, attained levels of 200 p.p.m. in the soil of the central reservation and at the edge of the carriageway. Pitfall traps were used to sample invertebrate populations at various distances from the carriageway and their lead concentrations were measured. Isopoda beside the road were found to contain 700 p.p.m. lead and Diplopoda 100 p.p.m., but despite this the species composition of the invertebrate communities did not seem to be affected by lead. Lead accumulation of this order was not reflected in small mammals, which had liver concentrations of only 10–15 p.p.m. and there was no evidence of further concentration at higher levels in the food chains.

The overall impression given by this conference is that the biologist is not yet in a position to solve many of the ecological problems posed by motorways.

Thiols and Membrane Structure

IN the next issue of *Nature New Biology* (November 14) Dupont and Hasselbach describe some extensive changes in the structure of the sarcoplasmic reticulum membrane that ensue when the ATPase is poisoned by thiol reagents. Titration with a mercurial, or with N-ethylmaleimide, reveals that there are ten to twelve reactive thiols per 10^5 molecular weight of protein in sarcoplasmic reticulum vesicles. The two types of ATPase activity that reside in this membrane respond differently to the elimination of free thiols: calcium-activated ATPase decreases linearly while four groups are reacted, whereas the basic magnesium-dependent ATPase evinces slight activation until five of the thiols have reacted, and thereafter falls off rapidly.

That extensive structural changes accompany the reaction of the thiol groups was at once apparent from an increase in turbidity, betokening aggregation of the vesicles. The changes are reflected in progressive alterations in intensity and position of the X-ray

spacings obtained from stacked oriented membranes. These are of relatively minor extent at low levels of substitution, but when more than about four thiols groups have been blocked the character of the X-ray pattern changes in a manner that can be interpreted in terms of the loss of the asymmetric structure which characterises the native membrane. The protein is not released but redistributed so that its average disposition with respect to the plane of the bilayer is symmetrical.

It seems then that substitution of the thiol groups causes the protein molecules to sink into the bilayer, either continuously or, according to a plausible hypothesis offered by the authors, on dissociation of a putative ATPase oligomer, which occurs when a critical number of thiol groups has been blocked. This will expose new protein surfaces and the nature of the interaction with the phospholipids in the bilayer will be changed in such a way as to favour a new and symmetrical structure.

Data Processing

from a Correspondent

ELECTRONIC data processing (EDP) has hitherto been used in very few herbaria, mostly for comparatively small projects, and its scope in the larger herbaria, particularly in Europe where most are located, has been hardly considered. An international meeting at the Royal Botanic Gardens, Kew, from October 3–6, had as its purposes to explore areas where EDP can help in herbaria and associated collections, and to ensure that future developments in the larger European herbaria shall be as far as possible compatible and their results internationally available. The NATO Eco-Sciences Panel acted as sponsor.

After introductions by Professor J. Heslop-Harrison, representing Kew, Professor J. G. Hawkes, chairman of the organising committee of the meeting, and Dr A. Rannestad, representing NATO, the contributions on the first day were chiefly focused on the scope of EDP in herbaria.

J. P. M. Brenan (Kew) described the priorities for EDP in a principal international herbarium, given that staff and finance are limiting factors. R. Ross (British Museum (Natural History)) spoke on the relationship between herbarium records and others, recognising that most important herbaria are associated with ancillary collections or living collections or, in the case of museums, with collections belonging to other disciplines. D. M. Henderson (Royal Botanic Garden, Edinburgh) discussed the problems presented in adapting label data on specimens for EDP purposes. The importance of this aspect is apparent from one of the resolutions passed at the end of this meeting. A. Gomez-Pompa and J. A. Toledo (National University of Mexico) and A. V. Hall (University of Cape Town) described operational schemes in their collections; the Mexican scheme involves a computerised record of the flora of Veracruz.

The general theme of the second day was to consider various EDP systems currently in use to give delegates an opportunity of asking questions and making comparisons. S. G. Shelter (Smithsonian Institution, Washington) described the ambitious and far-reaching Flora North America project—a data bank which uses the GIS system. The general collections at the Smithsonian Institution are recorded by a general-purpose information system called SELGEM, which was described by J. F. Mello. D. J. Rogers (University of Colorado) expounded the well-known TAXIR system devised for taxonomic purposes. The principles of general-purpose filing systems as devised

by the Information Retrieval Group of the Museums Association (IRGMA) were described by J. L. Cutbill (University of Cambridge). This system is basic to the programme for computerising the geological collections at the Sedgwick Museum in Cambridge. S. W. and D. M. Greene (British Antarctic Survey, Birmingham) described the principles behind the computerising of some 30,000 herbarium specimens in the Botanical Section of the British Antarctic Survey. F. H. Perring (Biological Records Centre, Monks Wood) spoke on EDP operations at the centre and made the point that many data can be handled by non-computerised methods, particularly microfilm and microfiche.

Many herbaria are linked with botanic gardens, the computerisation of whose living collections presents, of course, parallel problems. R. A. Brown (American Horticultural Society) described the recording system used at the Plant Records Center where a standardised system has been developed for recording and reporting information on plants cultivated in the principal North American botanical gardens and arboreta. At present the data files contain more than 170,000 records. This theme was later developed by J. Cullen who described the operation of the living plant record system at the Royal Botanic Garden, Edinburgh.

EDP systems in herbaria thus have an interface with similar systems for living collections. With the probable future international development of genetic resource centres, this is likely to increase in importance. Other discussions included the important application of herbarium label data to computerised mapping of plant distributions as developed in the National Herbarium of Canada in Ottawa. A further example was provided by D. B. Williams (British Museum (Natural History)) who discussed the link often found in principal museums between botanical and zoological collections, and described some of the special problems of the latter.

During the conference, recommendations were invited from participants, which were edited by a drafting committee, and put to discussion and vote by the delegates on the last day. The following is a summary of the principal resolutions which were passed, all of them either unanimously or by a very large majority:

(1) Data banks for plant collections should have a common minimal-standard set of descriptors based primarily on label data.

(2) A working party is to be set up to advise European herbaria on the set of descriptors referred to under (1).

(3) Individual herbaria should co-operate with national organisations, and national organisations should be en-

couraged to work through the International Committee on Museums (ICOM) towards widely-acceptable international standards of documentation.

(4) A recommendation will be sent to the Organising Committee of the International Botanical Congress, to be held in Leningrad in 1976, that EDP in taxonomic collections should be discussed and that consideration should be given to setting up a permanent International Commission to coordinate this.

(5) The working party will also consider systems and the possibility of establishing a pilot project in Europe.

(6) The working party will consider and advise on steps to form an international type-register.

(7) Relevant bodies should be encouraged to approach the appropriate United Nations agencies, particularly UNESCO and UNEP, to obtain funds for data banks on a national and international scale.

CELL MEMBRANE

Mobility of Surface Sites

from a Correspondent

MEMBRANE fluidity is certainly one of the most pervasive themes to have emerged in cell biology in recent years. The casual reader will be forgiven for believing that many cell biologists nowadays spend a considerable part of their time moving proteins around a sea of membrane lipid in a delightful molecular version of childhood pursuits. The carbohydrate binding proteins, for example concanavalin A which binds specifically to glucose or mannose-containing structures, are favourite probes for studying the mobility of molecules in the plane of the membrane. It is now widely believed that cell agglutination by con A results somehow from reorganisation of the cell surface to increase the ability of con A receptor sites to move together to form clusters and in the extreme case to 'cap' at one pole of the cell.

Tagging con A molecules with fluorescein allows application of a simple visual technique to the most sophisticated biological problems. A particularly interesting example has been described by Inbar, Ben-Bassat, Fibach and Sachs (*Proc. natn. Acad. Sci., U.S.A.*, **70**, 2577; 1973) who have found that the ability of mouse myeloid leukaemic cells to differentiate into phagocyte cells is related apparently to the fluid state of the surface membrane. Earlier work by Sachs and his colleagues had shown that normal undifferentiated haematopoietic cells transform *in vitro* into mature differentiated macrophages and granulocytes. Devel-

opment of macrophage and granulocyte colonies from single, normal undifferentiated cells requires constant interaction, presumably at the cell surface, with at least one specific mediator. The mediator is a protein with a molecular weight of about 65,000 that requires a small co-factor for activity (replaceable in certain conditions by adenine derivatives including cyclic AMP), and is released by various types of cells in culture into the tissue culture medium. The myeloid leukaemias contain two types of cells, only one of which can be induced to undergo normal differentiation. Again MG1 is needed but the leukaemic cells supply their own, as yet unidentified, co-factor. A clear difference between the two cell types was found by the ability only of the differentiation-competent cells to 'cap' con A molecules bound to their surface. In agreement with older results with normal lymphocytes, normal and virally transformed fibroblasts, clustering or 'cap' formation induced by cross-linking of membrane sites by con A was blocked by metabolic inhibitors, 'freezing' the membrane with aldehyde fixatives or simply by dropping the temperature. Again as often found in such cases, trypsinisation of differentiation-incompetent cells increased the mobility of con A binding sites and caps were formed.

Although thousands of similar observations have been described in a wide variety of systems, the molecular basis for increased mobility of surface sites is still obscure. A direct effect on the viscosity of the membrane lipids seems unlikely. The observation of Inbar *et al.* that 'cap' formation was helped by adding antibodies against con A, or of glycogen, after con A binding suggests that the surface sites were simply too widely spaced to form unaided the necessary cross-linked matrix with the lectin. Edelman *et al.* (*Proc. natn. Acad. Sci., U.S.A.*, **70**, 1442; 1973) suggest that the movement of surface sites is controlled by proteins attached to the inner cytoplasmic face of the surface membrane, the activity or concentration of which is altered. Certainly interaction between these internal proteins and external surface glycoproteins, that may span the cytoplasmic membrane, seems probable.

Whatever the true explanation turns out to be, however, the conclusion of Inbar *et al.* is that a difference in membrane fluidity of specific sites may explain differences in the ability of leukaemic cells to respond to the differentiation-inducing stimulus of MG1. It is now of some interest to correlate more directly the mobility of cellular MG1 receptor sites with the differentiation competence of those cells. Similarly one is curious about the behaviour of normally differentiation-incompetent

cells in an environment containing MG1 and low amounts of trypsin. Whatever the outcome there is no doubt that the experimental substance of this report will appear again and again in discussions of mechanisms controlling other facets of cell development.

INSECT METAMORPHOSIS

Competence to Moul

from our Insect Physiology Correspondent
It has long been known that when the 'imaginal disks' from young larvae of *Drosophila* are transplanted into larval hosts that are ready to pupate, they are unable to differentiate into adult structures. They gradually become competent for imaginal differentiation towards the end of the second instar, 50 to 70 h after hatching. The stimulus for the initiation of metamorphosis is a high concentration of the moulting hormone ecdysone.

In a short but interesting paper Mindek and Nöthiger (*J. Insect Physiol.*, **19**, 1711; 1973) describe a renewed attempt to discover the nature of the changes which result in the acquisition of competence. The experiments consisted in exposing eye-antennal disks removed from donor larvae 53 h old, which are completely incompetent for metamorphosis at this stage, and treating them in various ways before testing their competence by implantation into mature larvae, just before pupation at 120 h. Disks were maintained in adults receiving normal food, in adults 'starved' by feeding on sugar alone, or adults starved for a time and then given normal food. The results show that

competence to respond to ecdysone does not simply require a minimum number of cells (a 'critical mass'); nor is it determined by the time elapsed since the formation of the disk.

The authors conclude that cell divisions are the prerequisite for gaining new developmental possibilities and that a minimum number of cell divisions is needed to allow the necessary changes in the developmental programming of the cells.

PROTEIN SYNTHESIS

Control Mechanisms

from a Correspondent

EXPERIMENTS with mammalian cells in culture have revealed many diverse situations in which the rate of initiation of protein synthesis is greatly reduced. Besides the now classical example of rabbit reticulocytes deprived of haem, inhibition of initiation is seen when cells enter mitosis, are shifted to 42° C, starved of serum or an essential amino acid, infected with poliovirus or exposed to anaerobiosis. In some cases the inhibition is selective; more often the inhibition appears to affect all proteins to the same extent, but, apart from the inhibition of globin synthesis in the absence of haem, very little is known about the underlying control mechanisms. One reason for this is that extracts of cells other than the reticulocyte show almost no initiation on endogenous messenger RNA, so that normal and inhibited cells cannot easily be compared *in vitro*. This impasse has been present for a long time and only

Secondary Structure in an RNA Molecule

As soon as the primary structure of an RNA molecule is elucidated its discoverers set about the happy task of looking for possible secondary structures. When these are published they have tended to elicit alternative secondary structures from competing workers. How can a choice between these alternatives be made? The answer requires a means of assessing the free energy of a given structure and this in turn requires basic information concerning the free energy changes attendant on the formation of the various features—such as base pairs, hairpin loops, interior loops, bulges—which constitute the structure. Each new investigation of the thermodynamics of the secondary structure in oligonucleotides and other simple models leads to a refinement in these calculations.

In their latest paper Tinoco *et al.* (see *Nature New Biology* next Wednesday, November 14) amass recent results for

the sequence dependence of the free energy of formation of base pairs as well as that of various types of bulge and loop. The result is a table of free energy changes from which the total change on forming a given structure from the random coil state at 25° C can be calculated. Each entry in the table, it is claimed, is good to 10% but the uncertainty in the final figure may be somewhat greater; the authors also point out that the algorithm they describe does not take any account of tertiary structure. Nevertheless, it may serve to eliminate some of the more improbable models even if it does not allow a definitive assessment as to which is the most stable.

Tinoco *et al.* suggest that their work may lead to attempts to refine the theory and the experimental data on which it is based and even lead to investigation of temperature dependences which would be highly relevant to the interpretation of melting curves.

now, with a recent paper by Reichman and Penman (*Proc. natn. Acad. Sci., U.S.A.*, **70**, 2678; 1973), does it show any signs of being overcome.

Earlier work by Penman's group had shown that HeLa cells incubated at 42° C recover from the inhibition of initiation after 1–2 h. This recovery is prevented by actinomycin D, but not by cycloheximide, and, in fact, preincubation of cells at 37° C with low doses of this drug makes them less susceptible to inhibition of initiation at 42° C. These and other results suggested that when protein synthesis is slowed down, for example by high temperature, amino acid starvation or cycloheximide, the cells respond by making an RNA which promotes initiation. This RNA was predicted to have a half-life of about 1 h and to be rate-limiting for initiation in certain conditions.

When Reichman and Penman first tested extracts of normal HeLa cells for initiation *in vitro* they, like many others before, found very low activity, as measured by the pactamycin-sensitive incorporation of (³⁵S)-methionine into the N-terminal position of polysomal-bound nascent protein at 25° C. Bearing the earlier results in mind, however, they preincubated their cells for up to 4 h in the presence of a low concentration of cycloheximide and then prepared cell-free extracts, which showed up to six times more initiation. This increase in initiating capacity was prevented by preincubation with cycloheximide and actinomycin D, but was obtained when cells were preincubated in the absence of an essential amino acid. Developing the earlier model, Reichman and Penman suggest that the factor synthesised by intact cells in response to a reduction in protein synthesis is also the factor which is rate limiting for initiation in cell-free extracts, but they are somewhat more cautious in predicting that the factor is an RNA species. Although preliminary, these results open up exciting new possibilities for studying the control of initiation in mammalian cells.

PEAT

Origin of Palsa Mounds

from our Plant Ecology Correspondent

THE energy exchange properties of palsa mounds is the subject of a recent paper by Railton and Sparling (*Can. J. Bot.*, **51**, 1037; 1973), who provide a new approach towards understanding these mounds of peat with frozen cores which are abundant in the mires of high latitudes.

The word 'palsa' originated in Lapland and most of the research which has been undertaken on these features has come from Finland and the Soviet Union. In Finland palsas are only

found north of the coniferous forest limit in areas with a mean annual air temperature of –1° C and an annual precipitation of less than 400 mm. The palsas of that region are often irregularly shaped and may attain lengths of 200 m.

In the search for an explanation of the origin of these phenomena an initial question requiring solution is the start of their formation. By means of pollen analysis accompanied by radiocarbon dating, Salmi (*Proc. 3rd Int. Peat Congr., Quebec*, 182; 1968) came to the conclusion that palsas in Lapland fall into two groups, one of which began formation around 5000 BC and the other at about 3000 BC. The age of a palsa, however, did not seem to be related to its size. Other Finnish workers have found palsas of more recent origin and are less inclined to regard palsas as relics of past climates.

Railton and Sparling align themselves with those who believe in a continuous cycle of formation and erosion of the palsa mounds. They base this belief on their observation that all size classes of palsas are represented in their study area in northern Ontario and also that these classes exhibit a complete intergradation. They do not attempt to justify the assumption that palsa size is a function of palsa age, an assumption which appears rather tenuous in the light of Salmi's work.

They do, however, demonstrate some interesting relationships between palsa size and vegetation cover. Palsas containing a high percentage cover of *Sphagnum fuscum* were mainly less than 60 cm high and less than 7 m long. Lichen-rich communities with, for example, *Cladonia alpestris*, *C. floerkeana*, *C. rangiferina*, were found mainly on mounds greater than 60 cm in height. On the basis of these observations Railton and Sparling suggest that palsas develop continuously, and probably originate as *Sphagnum fuscum* hummocks on the mire surface. They become invaded and dominated by lichens during growth and finally erode and collapse. It is possible that the changes in heat exchange properties associated with the development of a lichen cover could account for the process of palsa development.

To test the feasibility of such a model Railton and Sparling measured the energy exchange properties of the different vegetation types. Total albedo was least on the general mire surface, increased in the *Sphagnum fuscum* hummocks, was greater still in hummocks dominated by lichens and reached a maximum in mature palsas. The obvious question which follows is whether these increases in albedo associated with palsa development are adequate to account for their negative heat budget and hence their growth. A

consideration of the total energy budgets of palsas led Railton and Sparling to the conclusion that the differences in albedo were not sufficiently great when compared with other energy exchange factors, and they are inclined to regard changes in the soil heat flux as being of greater importance. Thermal conductivity in peat decreases as the peat dries. Drier peat on the top of hummocks would lose heat less rapidly in summer, so helping to preserve the permafrost core. If this is so, then the lichen cover so often associated with mature palsas should be regarded as a product of mound development rather than a cause.

PLATE TECTONICS

Subduction Heating

from our Geomagnetism Correspondent

ACCORDING to the hypotheses of the new global tectonics, oceanic trenches, where colder oceanic lithosphere descends into the warmer underlying mantle, should be regions of relatively low surface heat flow; and this prediction is generally confirmed by observation. But observation also shows that behind oceanic trenches (that is, on the side of the trench opposite the spreading ocean floor) the heat flow is above average and associated with the volcanic island arcs. For some years after the basic principles of seafloor spreading and mantle convection had been mapped out, the causes of the volcanism and high heat flow, though clearly related in some way to global tectonic processes, remained obscure, and even today are far from being completely understood. In 1968, however, Oxburgh and Turcotte (*Nature*, **216**, 1041; 1968) and McKenzie and Sclater (*J. geophys. Res.*, **73**, 3173; 1968) made a significant advance by proposing independently that the origin of behind-trench phenomena lies in frictional heating on the slip zone between the descending lithospheric plate and the overlying mantle.

Such frictional heating certainly seems to be able to account for the surface observations behind oceanic trenches; and so several workers have attempted in various ways to calculate the subsurface temperature distribution in the descending plate. Turcotte and Oxburgh (*Phys. Earth planet. Inter.*, **1**, 381; 1968) and McKenzie (*Geophys. J.*, **18**, 1; 1969), for example, assumed in their calculations that the temperature is constant at the slip zone, whereas in later work McKenzie (*Tectonophysics*, **10**, 357; 1970) and Griggs (in *The Nature of the Solid Earth*, McGraw-Hill; 1972) assumed the slip zone temperature to be equal to that of an undisturbed mantle. Then again, Oxburgh and Turcotte (*Bull. geol. Soc. Am.*, **81**, 1665; 1970) assumed that the

shear stress on the slip zone decreases with depth, whereas Minear and Toksöz (see, for example, *Tectonophysics*, **10**, 367; 1970) incorporated shear stress heating on both boundaries of the descending slab. But as Turcotte and Schubert (*J. geophys. Res.*, **78**, 5876; 1973) now point out, in these calculations little or no attention has been given to the positions of the volcanic chains behind ocean trenches—that is, to the geometry of the very system resulting from the thermal phenomena under investigation.

With the upsurge in geophysical activity during the past decade or so, many such volcanic chains have become quite familiar; and examples include the Kurile Islands, the West Indies, the Volcano and Mariana islands, Sumatra and Java, the Tonga and Kermadec islands and the New Hebrides. Turcotte and Schubert now propose that all these volcanoes lie above the points on the various slip zones where melting of the descending crust takes place. This assumes, of course, that the magma produced at the slip zone rises vertically to the surface, presumably under the buoyancy force arising from low magma density. Because the hydrostatic pressure is at least an order of magnitude greater than the ultimate strength of the rock at the depths of 100–200 km where the magma forms, the magma will probably move through the rock by the rock's plastic deformation rather than through a fracture; and in the absence of any other preferred direction, this movement will be vertically upwards. Accepting this geometry, the positions of the volcanoes may then be related to a specific process (melting) taking place on the slip zone and thus give an additional fixed point in calculating the subsurface temperature distribution.

Turcotte and Schubert develop two different models of heating on the slip zone. In the first, the shear stress along the slip zone is assumed to be constant down to the depth at which the basalt solidus temperature is reached; and thereafter the temperature at the slip zone remains at the basalt solidus (which increases with depth, of course). In the second model, the shear stress on the slip zone is allowed to increase linearly with depth to the point at which the solidus temperature is reached; and again, temperatures at greater depths are assumed fixed at the basalt solidus. Since the linearly increasing shear stress is equivalent to a constant coefficient of friction on the slip zone, the two models may be characterised, respectively, as 'constant shear stress' and 'constant friction'.

To cut a long physico-mathematical story short, what emerges from the Turcotte-Schubert analysis is an equation for each model which may be com-

pared with observation. In the case of the constant shear stress model, the operative equation gives shear stress in terms of the thermal properties of the system, its geometry (including the vertical distance from the volcanoes to the slip zone) and the velocity of the descending plate. Feeding in observational values for eight individual trenches gives shear stresses in the range 1.18–1.60 kbar, with a mean of 1.35 kbar and a standard deviation of 10%. For the constant friction model, the operative equation is for coefficient of friction in similar terms but with the addition of the mean density above the slip zone. Frictional coefficients calculated from observational parameters lie in the range 0.033–0.077, with a mean of 0.054 and a standard deviation of 24%.

In attempting to decide between the two models, these results are perhaps less clear than might have been hoped. Most of the volcano to slip zone distances lie between 100 and 120 km with errors in locating the slip zone estimated to be $\pm 20\%$. More variable distances would clearly have given a more convincing test for the constancy of either the shear stress or the frictional coefficient. Exceptionally, however, the volcano-slip zone distance for the New Hebrides system is 200 km; and the calculated shear stress at 1.31 kbar is much closer to the mean shear stress than the frictional coefficient of

0.033 is to the mean frictional coefficient. This, together with the smaller scatter of the shear stress values for the eight trench systems, leads Turcotte and Schubert to suggest that the constant shear stress model is probably the better of the two, although they are unwilling to commit themselves to a definite conclusion.

By extending their calculations to depths below the melting zone, Turcotte and Schubert go on to determine the thermal structure of the descending plate consistent with each model, on the assumption that the descending plate is largely olivine and thus that heating due to the olivine-spinel phase transformation, as well as adiabatic heating, is important. Other things being equal, the near-surface heating on the slip zone is somewhat stronger for constant shear stress than for constant friction, although for constant friction melting occurs at a slightly shallower depth. Apart from that, however, the temperature profiles are very similar. Finally, the temperatures on the slip zone are in fairly good agreement with those obtained by Toksöz *et al.* (*J. geophys. Res.*, **76**, 1113; 1971) but the minimum temperatures in the descending plate are about a factor of two lower. Turcotte and Schubert thus agree with others that the plate temperatures obtained by Toksöz *et al.* were probably too high, although the reason for the discrepancy is not clear.

Search for the Superheavies

ONE of the more interesting developments in nuclear physics recently has been the prediction by theorists of the existence of superheavy elements. This island of elements, with atomic numbers in the region of 114, lies significantly beyond the heaviest nuclei (numbers 104 and 105) which have so far been artificially produced and owes its possible existence to the extra binding energy and stability associated with the doubly closed nucleon shells at 114 protons and 184 neutrons.

Prediction of the half lives of these elements is particularly difficult, but some calculations have suggested values as long as 10^9 to 10^{15} years, implying that they might be found in natural materials on Earth. Several searches for superheavy elements in natural ores, minerals and so on have already been carried out and the results from one of the most complete are to be published in next Monday's *Nature Physical Science* (November 12).

The work has been carried out by a large group based on the Oak Ridge National Laboratory in Tennessee. The technique which they use relies on the prediction that superheavy elements are very likely to undergo spontaneous

fission and could emit an average of ten neutrons for each fission event, to be compared with values of less than four for presently known spontaneously fissioning nuclides. The neutron multiplicity counter they use records events in which either three, or more than three, neutrons are detected.

The technique has the advantage that bulky samples can be used and no chemical processing is necessary. Materials investigated range from mineral and ore samples, through flue dust and other samples collected during ore processing, to manganese modules and 60 million-year-old sharks' teeth from a depth of 15,000 foot in the Pacific Ocean and meteorites from Mexico.

In spite of this very wide range of samples no evidence was found for spontaneous fission events accompanied by large numbers of neutrons. It seems then unlikely that superheavy elements will be found naturally on Earth and that physicists will have to await attempts to produce them with the heavy ion accelerators now being planned and under construction. The search for the superheavies will no doubt continue.

LIQUID HELIUM

New Excitation

from our Condensed Matter Correspondent

A NEW type of elementary excitation, christened the ^3He roton, may exist in liquid ^3He - ^4He mixtures according to a calculation by Stephen of Rutgers University and Mittag of the University of Miami, published in a recent issue of *Physical Review Letters* (31, 923; 1973) and based on a suggestion made by L. P. Pitaevski during the United States-Soviet symposium on condensed matter at Berkeley in May.

Below its superfluid transition temperature T_λ , liquid ^4He can be regarded as an inert superfluid background in which exists a gas of elementary excitations carrying all the entropy of the liquid and thus being responsible for its thermodynamic properties. At zero temperature there are no excitations but, as T is increased, the density of the excitations, known as the normal fluid density (ρ_n), increases until at T_λ it is equal to the overall density of the whole liquid. In pure liquid ^4He , except very close to T_λ , there are two distinct types of elementary excitation: at small momenta, the energy ϵ of an excitation is directly proportional to its momentum p , and it is thus a phonon, or quantum of sound; at higher momenta there is a minimum in the $\epsilon(p)$ curve, and the excitations, all of energy close to Δ , existing in this region are known as rotons. Their physical nature is not entirely clear, but they may, perhaps, be the microscopic quantum mechanical analogue of classical vortex rings.

The addition of small proportions of ^3He profoundly affects the properties of the liquid. The ^4He atoms act as additional excitations, and thus increase the normal fluid density and contribute to the thermodynamic properties. Measurements of the heat capacity and wave velocity at very low temperatures have shown that a ^3He atom behaves very much like a free particle in that its energy varies as the square of its momentum, but with a modified mass m_3^* to take account of the superfluid background through which it moves.

When Sobolev and Esel'son made direct experimental measurements of ρ_n (*Zh. éksp. teor. Fiz.*, 60, 240; 1971) they found that ρ_n seemed to increase with ^3He concentration much more rapidly than expected. Since the contribution to ρ_n from the ^3He atoms is just $n_3 m_3^*$ where n_3 is their number density, and that from phonons at any given temperature depends only on the velocity of sound which does not change much with n_3 , it was concluded that the effect must be associated with a decrease in the roton energy Δ . Recent experimental measurements of Δ by Surko and Slusher of Bell Laboratories, and independently by Woerner, Rock-

well and Greytak of MIT, both using light scattering techniques and reported in the same issue of *Physical Review Letters* (30, 1111 and 1114; 1973) have shown, however, that Δ does not change significantly with ^3He concentration.

It was this apparent contradiction in the experimental evidence for the value of Δ which led Pitaevski to suggest that the $\epsilon(p)$ curve for ^3He excitations in liquid ^4He might also display a minimum, much like the roton minimum. If the value of ϵ at the minimum were less than Δ , then there would clearly be a possibility of resolving the conflict.

Stephen and Mittag have now carried out a detailed calculation, using the same theoretical approach as in the original Feynman-Cohen derivation of the $\epsilon(p)$ spectrum for pure ^4He . As Pitaevski had surmised, they find that the ^3He excitation spectrum does indeed display a minimum, that this occurs at an energy lower than Δ , and that it is thus possible to reconcile quantitatively, within experimental error, the measured values of ρ_n with those of Δ . The probable reason that ^3He excitations near the minimum, referred to as ^3He rotons, were not also observed in the light scattering experiments is, the authors suggest, that the relative intensity of the scattering associated with rotons and ^3He rotons is proportional to the square of the ratio of the ^4He and ^3He number densities, so that, because the proportion of ^3He was always less than 30%, the former scattering process would have been strongly dominant.

Convincing confirmation that ^3He rotons really do exist, however, must probably await the results of further light scattering experiments.

CRYSTAL GROWTH

Crystallite Coalescence

from our Materials Science Correspondent
MASSON and his colleagues in Marseilles have published a series of beautifully executed electron microscope studies of the early stages of condensation of metallic films on ionic substrates; the strikingly novel finding from this work was that small crystallites can wander about in a two-dimensional walk, and, above a certain temperature, the migrating crystallites will coalesce when they touch by chance in the course of their wanderings. These findings have been summarised before (*Nature phys. Sci.*, 241, 1; 1973).

Now some theoretical implications of the Marseilles findings have been worked out by Robertson (*J. appl. Phys.*, 44, 3924; 1973) in a stimulating paper. He starts from the observation (by the Marseilles team and others) that whereas crystallites do in fact coalesce at elevated temperatures (for example, above

PHYTOPATHOLOGY

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Whatever they are doing now they can probably all be found out and tracked down in this directory. Within its covers 11,000 practitioners of the healing vegetable arts are listed under their countries of origin, together with address, telephone number and often details of professional interests. It is available (price \$12) from the editor at the University of Florida, IFAS, AREC, PO Box 1088, Lake Alfred, Florida 33850.

150° C in KCl), they do not at lower temperatures, although other evidence indicates that they do migrate at these lower temperatures. Robertson analyses the predicted coalescence kinetics in terms of migration rates and collision probabilities (which themselves depend on crystallite sizes). It turns out that there should be extensive coalescence under conditions when in fact none is experimentally observed.

Robertson speculates on possible reasons why collisions should not result in coalescence. Three ideas are put forward: the crystallites may in various ways pick up electrostatic charges or the substrate surface may be contaminated; either form of disturbance is known to inhibit coalescence of colloids suspended in fluids. The third source of disturbance, examined in more detail, is the buildup of misfit stresses between substrate and crystallites, and Robertson indicates that the possibly sharp dependence of misfit stress on crystallite size could lead to either enhancement or hindrance of coalescence. He concludes by claiming that the very process of crystallite migration is not yet established beyond all doubt, and that this is a research topic deserving of much more attention, since it may force a complete revision of the classical concept of heterogeneous nucleation and growth.

Irreversibility as a Symmetry-breaking Process

I. PRIGOGINE

Université Libre de Bruxelles, Brussels, Belgium, and Center for Statistical Mechanics and Thermodynamics, University of Texas at Austin, Texas

This article is based on the 1972 Bourke lectures of the Faraday Division of the Chemical Society.

It is just one hundred years ago that Boltzmann published his celebrated paper "Further Studies on Thermal Equilibrium between Gas Molecules"¹, in which he formulated his kinetic equation for the velocity distribution $f(\mathbf{v}, t)$ of dilute gases²

$$\partial f / \partial t = \int d\Omega \int d\mathbf{v} \, \sigma(\Omega) |\mathbf{v}_1 - \mathbf{v}| (f_1' f' - f f_1) \quad (1)$$

He then introduced the quantity $\mathcal{H}_B$ defined through

$$\mathcal{H}_B = \int d\mathbf{v} f \log f \quad (2)$$

and established as a consequence of equation (1) the inequality

$$d\mathcal{H}_B / dt \leq 0 \quad (3)$$

This basic inequality led him to the first microscopic model of entropy

$$S = -k\mathcal{H}_B \quad (4)$$

Boltzmann used physical intuitive arguments to obtain equation (1). He emphasized that the Second Law of Thermodynamics deals with large systems and that he therefore felt justified in adding to the laws of dynamics additional elements based on probability calculus.

Why are Boltzmann's investigations so central? They link together various levels of description of the physical world which had been introduced independently: classical or quantum dynamics, stochastic theory and thermodynamics.

Boltzmann's $\mathcal{H}$ theorem has led to a strange situation, perhaps unique in the history of science. On one side Boltzmann's kinetic equation has been applied successfully to many physical phenomena such as the calculation of transport properties of gases². More important still is the fact that computations based on molecular dynamics have entirely validated Boltzmann's predictions³⁻⁵. Such computer experiments show that indeed Boltzmann's quantity $\mathcal{H}_B$ decreases with time. (Because of the finite size of the sample, fluctuations appear but they decrease when the size of the sample is increased.)

These numerical experiments are very important, for they show that using only the laws of classical dynamics inequality (3) is satisfied for sufficiently large assemblies of interacting particles. In other words, the effects described by Boltzmann's kinetic equation (1) are in agreement with the results of numerical integrations based on classical dynamics. This gives one a strong incentive to investigate the conditions in which a dynamic system may present "dissipative" properties such as described by inequality (3), without introducing additional non-mechanical elements. I shall show that it is now possible to present a rigorous "mechanical theory" of irreversibility. Here I shall present only a very concise account of some of the chief ideas, omitting all proofs. More information can be found in refs 6, 7 and 8.

Time Reversal Symmetry

I shall start from classical or quantum dynamics as expressed by Hamilton's equations of motion

$$dq/dt = \partial H / \partial p \quad dp/dt = -\partial H / \partial q \quad (5)$$

or Schrödinger's equation

$$i\partial\psi/\partial t = H\psi \quad (6)$$

Both descriptions may be unified through the Liouville-von

Neumann equation for the distribution function (or the density matrix⁹)

$$i\partial\rho/\partial t = L\rho \quad (7)$$

with

$$L\rho = -i\{H, \rho\} \text{ Poisson bracket} \\ [H, \rho] - \text{commutator} \quad (8)$$

In both cases L is a hermitian operator (better called a super-operator because it acts on matrices in the quantum mechanical case, see refs 7 and 8). Therefore

$$L = L^\dagger \quad (9)$$

A basic feature of equation (7) is its " Lt invariance". Indeed, under the combined effect of the two operations

$$\begin{cases} L \rightarrow -L \\ t \rightarrow -t \end{cases} \quad (10)$$

equation (7) remains invariant. Note that if L is a possible Liouville operator so is $-L$. The " Lt invariance" expresses in a concise fashion the time reversal symmetry.

Now in spite of the " Lt invariance" some types of irreversible processes are still possible. For the example of a single particle enclosed in a large box the Hamiltonian is

$$H = \mathbf{p}^2 / 2m \quad (11)$$

and equation (7) becomes

$$\partial\rho/\partial t + (\mathbf{p}/m) \cdot \partial\rho/\partial\mathbf{r} = 0 \quad (12)$$

In rather general conditions the density in coordinate space obtained from ρ through integration over the velocities, will become uniform in the box both for $t \rightarrow +\infty$ and for $t \rightarrow -\infty$ (see ref. 9, chapter 8). This is because the spectrum of the operator is continuous (see refs 9-12). Indeed, as can be easily verified (ref. 9, chapter 1) its eigenvalues are

$$\lambda = \mathbf{k} \cdot (\mathbf{1}/m)\mathbf{p} \quad (13)$$

where $\mathbf{k}$ is a real vector the value of which is determined by the boundary conditions.

Less trivial examples have also been considered. For example, one may study chains of harmonic oscillators and follow how equilibrium between kinetic and potential energy is achieved in the limit of large lattices¹³⁻¹⁸. In none of these cases, however, do quantities such as Boltzmann's collision operator or thermodynamic transport coefficients appear. This obviously requires stronger conditions than the continuous L spectrum. This conclusion may already be inferred from the structure of phenomenological equations such as the Fourier equation

$$\partial T / \partial t = \kappa \partial^2 T / \partial x^2 \quad (14)$$

As the heat conductivity κ is a positive quantity its sign cannot be reversed. Therefore the time inversion $t \rightarrow -t$ leads to the different equation

$$\partial T / \partial t = -\kappa \partial^2 T / \partial x^2 \quad (15)$$

This makes a pair of equations in each of which the direction of time plays a different role (in equation (14) the temperature becomes uniform for $t \rightarrow +\infty$, in equation (15) it was uniform for $t \rightarrow -\infty$); by contrast the single equation (12) predicts uniformity for distribution in coordinate space for both $t \rightarrow -\infty$ and $t \rightarrow +\infty$. The time reversal symmetry present in the dynamical description of equation (7) has been broken in equations (14) and (15).

One may say that the Second Law of Thermodynamics

$$dS/dt \geq 0 \quad (16)$$

summarizes in a single inequality all laws such as Fourier's law, friction phenomena and so on which are described by phenomenological equations involving a privileged direction of

time. This is the situation we associate with "thermodynamic irreversibility" and it is with this type of irreversibility that I am concerned here.

To return to the central problem: What are the conditions that have to be imposed on dynamics to obtain this behaviour? I believe that we can now give a precise answer to this question and show that the symmetry breaking is due to the possibility of analytical continuation for well defined dynamic quantities.

Dynamics and Dissipation

I shall first write the formal solution of the parabolic equation (7) in terms of the resolvent of the operator L (see ref. 9).

$$\rho(t) = \exp(-iLt)\rho(0) = (2\pi i)^{-1} \int_c dz \exp(-izt)(1/L-z)\rho(0) \quad (17)$$

The importance of this formal representation of $\rho(t)$ in terms of the resolvent comes from the fact that this representation incorporates causality (see refs 19 and 20). Indeed the contour c is a parallel to the real axis but has to be taken above or below it according to whether "retarded" solutions for $t \geq 0$ or "advanced" solutions for $t \leq 0$ are considered. The solution $\rho(t)$ is determined both by the differential equation (7) and the choice of the contour.

The formalism which I shall introduce now permits a discussion of the behaviour of the resolvent in the complex plane. The first step is to define orthogonal hermitian projection operators P, Q such that

$$P + Q = 1 \quad (18)$$

The choice of these operators fixes the "language" in which the results are formulated. I shall consider specific examples later. Generally P is chosen to project onto the diagonal elements in the representation in which some model Hamiltonian H_0 is diagonal (using here quantum mechanical notations)

$$\langle m|P\rho|n\rangle = \langle m|\rho|m\rangle\delta_{m,n} \quad (19)$$

As diagonal elements give no information about correlations between particles $P\rho$ is called the "vacuum of correlations".

Using P and Q it is easy to verify the following identity for the resolvent²¹⁻²³

$$\frac{1}{L-z} = \{P + \mathcal{G}(z)\} \frac{1}{PLP + \Psi(z) - z} \{P + \mathcal{D}(z)\} - \mathcal{D}(z) \quad (20)$$

with

$$\Psi(z) = -PLQ \frac{1}{QLQ - z} QLP \quad (21)$$

$$\mathcal{D}(z) = -PLQ \frac{1}{QLQ - z} \quad (22)$$

$$\mathcal{G}(z) = -\frac{1}{QLQ - z} QLP \quad (23)$$

$$\mathcal{D}(z) = -\frac{1}{QLQ - z} \quad (24)$$

These are the basic operators in the formulation of non-equilibrium statistical mechanics. Of special importance is the collision operator $\Psi(z)$: reading equation (21) from right to left it corresponds to a transition from the vacuum of correlations followed by a dynamical evolution in the correlation space and finally followed by a return to the vacuum of correlations. The operator $\mathcal{D}(z)$ corresponds to a transition $P \leftarrow Q$ and is therefore called the destruction (of correlations) operator, similarly $\mathcal{G}(z)$ is the creation (of correlations) operator. All these operators depend both on the dynamics of the system (through the Liouville operator L) and on the language used (through the projection operators P and Q).

The important point is that through the introduction of these operators one can study the limit for $z \rightarrow +i\epsilon$ and the analytic continuation of the resolvent (20).

Insertion of equation (20) into equation (17) leads to an integrodifferential equation for the retarded solution. This equation is generally called the "master equation"⁹ (for recent

applications see refs 24 and 25). Neglecting non-local and memory effects the master equation takes the simple form for the diagonal elements of the density matrix^{8,9}

$$i\partial\rho_0/\partial t = \Psi(0)\rho_0 \quad (25)$$

with

$$\Psi(0) = \lim_{z \rightarrow +i\epsilon} -PLQ \frac{1}{QLQ - z} QLP \quad (26)$$

(P has been chosen in such a way that PLP vanishes.) The collision operator has a specially simple physical meaning in the case of potential scattering. The matrix elements (in momentum representation) $\langle p|\Psi(0)|p'\rangle$ give then directly the scattering cross section for the transition $p \leftarrow p'$ (ref. 26).

In the limit of large systems, the sum over the intermediate states Q (the "correlations") involves an integration. Therefore a formal representation of equation (26) is given by²⁷

$$\begin{aligned} -i\Psi(0) = & -\pi PLQ\delta(QLQ)QLP \\ & + iPLQ \frac{1}{QLQ} QLP \end{aligned} \quad (27)$$

where the second term is understood as a principal part. If this formal expression is meaningful and each of the two terms exists, it follows that

(a) $-i\Psi(0)$ is split into an even and an odd part with respect to L inversion

$$\Psi(0) = \overset{\circ}{\Psi}(0) + \overset{\circ}{\Psi}(0) \quad (28)$$

(b) the even part is a negative operator

$$-i\overset{\circ}{\Psi}(0) \leq 0 \quad (29)$$

(c) the even part $\overset{\circ}{\Psi}(0)$ is hermitian while the odd part is anti-hermitian.

All these three properties are quite important. They solve in fact the three fundamental problems which have always faced non equilibrium statistical mechanics. First of all if the even part $\overset{\circ}{\Psi}(0)$ exists, the master equation (25) obviously loses the Lt invariance. After Lt inversion one obtains

$$i\partial\rho_0/\partial t = [-\overset{\circ}{\Psi}(0) + \overset{\circ}{\Psi}(0)]\rho_0 \quad (30)$$

This is precisely what would have resulted if in equation (17) the contour below the real axis had been taken and the "advanced" solutions constructed. The situation is quite similar to that discussed in the context of the Fourier equation (see equations (14) and (15)).

In the second place inequality (29) shows that the even part may be considered as expressing a "damping" which occurs in the future in equation (25) and in the past in equation (30) (for more details see ref. 6). Therefore inequality (29) contains in a nutshell the Second Law of Thermodynamics, equation (16). More precisely because of equation (29) a microscopic model of entropy can be constructed such that this entropy can only increase (or remain constant) for all initial value problem (see ref. 8 and I. P. and F. Henin, to be published). I shall come back to this question later.

Finally the third property (hermiticity of $i\Psi(0)$) permits it to be given, at least in simple cases, an interpretation in terms of transition probabilities in the sense of a Markoff chain^{8,28}. It is this property which leads from the initial dynamic description to a description in terms of stochastic processes.

One can see how deeply dynamics is altered when the collision operator $\Psi(0)$ does not vanish. The appearance of an even part in $\Psi(0)$ leads to a radical change in the dynamic description of the system. It should be noted that the discussion has been based on equation (25) rather than the general master equation. A general discussion will be presented later.

Dynamic Systems with Broken Symmetry

The basic importance of the even part $\overset{\circ}{\Psi}(0)$ leads me to introduce the following classification: (a) $\overset{\circ}{\Psi}(0)$ vanishes; this

is the situation for quantum systems with a discrete non-degenerate spectrum (then $z=0$ in equation (26) does not belong to the spectrum of QLQ); (b) $\dot{\Psi}(z)$ becomes undefined when the limiting process $z \rightarrow +i\epsilon$ is performed. This may be the case for quantum system with discrete but degenerate spectrum (for examples see refs 29 and 30). This situation also occurs in classical dynamics when gravitational forces are involved^{31,32}; (c) finally, there is the situation in which $-i\dot{\Psi}(0)$ is well defined and negative

$$-i\dot{\Psi}(0) \leq 0 \quad (31)$$

This is the dynamic dissipativity condition. (This terminology agrees with the common mathematical terminology³³.) When satisfied it implies a qualitative change in the structure of the invariants of the system and it is a necessary condition for a thermodynamic type of behaviour. As already noted, inequality (31) cannot occur unless the spectrum of L is continuous. At least for quantum systems this is only possible in the limit of an infinite volume and eventually also of an infinite number of particles (the so-called thermodynamic limit).

For scattering these three cases correspond, respectively, to the situations where the S matrix is unity (no scattering), where the scattering cross section diverges and finally to the situation where the cross section is different from zero, when it is necessarily positive. The dissipativity condition also applies, however, to much more general situations. Examples of N body dissipative systems have long been known⁹. The operator $\Psi(0)$ has been calculated explicitly for systems such as weakly coupled gases or slightly anharmonic solids. In all these cases it is easy to verify that in accordance with our general statement $-i\dot{\Psi}(0)$ has an even part, is negative and hermitian. For such systems the dissipativity condition has a very simple meaning—that there are processes which conserve energy and momentum such that the integral involved in $\dot{\Psi}(0)$ (see equation (17) and remarks earlier in this article) does not vanish. This was discussed in detail some years ago (see ref. 9, chapter 14). This interpretation applies, however, only to very simple situations.

The construction of $i\dot{\Psi}(0)$ may be extended to strongly coupled system using formal perturbation techniques⁸. It is also very instructive to study simple models where a rigorous, non-perturbative approach is possible.

A simple example is given by the Hamiltonian

$$H = \sum_{m,n=-N}^N |m\rangle H_{m,n} \langle n| \quad (32)$$

where $H_{m,n}$ is a hermitian, Toeplitz form characterized by the properties

$$H_{m,n}^N = H_{m-n}^N \quad (33)$$

These systems have recently been studied carefully by Gibberd and Stey, Stey^{29,30} and Grecos³⁴. In the limit $N \rightarrow \infty$ they satisfy rigorously the dissipativity condition (31).

A second example is Friedrichs's model^{28,35-39}. The Hamiltonian is then of the form

$$H = H_0 + \lambda V \quad (34)$$

where H_0 has a continuous spectrum and an embedded discrete eigenvalue ω_0 coupled through the λV perturbation.

The Friedrichs model is related to various models investigated in the literature such as the Wigner-Weisskopf model for a two-level atom, the Lee model in field theory and so on.

As in the case of the Toeplitz forms, the Friedrichs model is soluble, as the transformation which diagonalizes H can be obtained. Because of the continuous nature of the spectrum, H will admit in general both eigenfunctions and eigendistributions (containing δ functions as well as principal parts).

We may choose³⁶ P so that Pp is the probability of finding the unstable (discrete) state excited at time t . Here again the resolvent of the von Neumann operator as well as $\Psi(z)$ can be obtained by a direct calculation using equations (20) and

(21). It turns out that

$$\Psi(0) = \dot{\Psi}(0) = \left[-\frac{1}{2i\pi} \int_{-\infty}^{+\infty} dx \eta^+(x) \eta^-(x) \right]^{-1} \quad (35)$$

Here $\eta^\pm$ are the limiting values on the real axis of the analytic function

$$\eta(z) = \omega_0 - z - \lambda^2 \int_0^\infty d\omega |\nu(\omega)|^2 / (\omega - z) \quad (36)$$

I assume that the continuous spectrum of H_0 and thus of H extends over the positive real axis; $\nu(\omega)$ measures the coupling between ω_0 and the continuous spectrum.

Because in this case P is a one-dimensional projection, $\Psi(0)$ as given by equation (35) is a number (while in the Toeplitz case Ψ is an operator represented by a matrix).

Using equation (36) we have shown³⁶ that $\dot{\Psi}$ does not vanish if the coupling parameter λ is smaller than a critical value, a condition which implies that H has no point spectrum corresponding to a bound state; $i\dot{\Psi}$ is essentially the inverse of the life time of the unstable state. These examples establish rigorously the existence of dynamic systems with broken Lt symmetry.

Invariants of Motion and Dissipation

One has to expect a close connexion between the non-vanishing of the collision operator $\Psi(0)$ and the problem of the invariants of motion.

I shall consider a Hamiltonian H together with the projection operator P (see equation (18)) and write

$$H = PH + QH = H_0 + \lambda V \quad (37)$$

This relation defines the "unperturbed" Hamiltonian H_0 . Now suppose that the problem of invariants for PH has been solved; every observable $P\Phi$ commutes with PH and is an invariant of motion. The basic problem will be to study the possible relation between the invariants of H and of PH . Now suppose that Φ is an invariant for the dynamical system. Then

$$L\Phi = 0 \quad (38)$$

The invariants Φ may be classified into regular or singular^{36,37,40}. (Briefly, singular invariants are such that their off-diagonal part $Q\Phi$ does not satisfy the regularity conditions necessary to include it in a Cauchy integral). If both the collision operator $\Psi(z)$ and the creation operator $\mathcal{C}(z)$ (see equation (23)) exist in the limit $z \rightarrow +i\epsilon$, then starting from equation (38) a few manipulations show that regular invariants satisfy the conditions^{36,40,42}

$$\Psi(0)P\Phi = 0 \quad (39)$$

$$Q\Phi = \mathcal{C}(0)P\Phi \quad (40)$$

showing the essential importance of the collision operator. If it were to vanish the result would simply be

$$Q\Phi = \mathcal{C}(0)P\Phi \quad (41)$$

To each invariant Φ_0 of PH , then, corresponds the invariant Φ_0 , $\mathcal{C}(0)\Phi_0$ of H . If, however, $\Psi(0)$ does not vanish then the conditions for regular invariants (39) and (40) are much more restrictive as $P\Phi$ has then to be an eigenfunction of the collision operator corresponding to a zero eigenvalue. Obviously this destroys the one-to-one correspondence between the invariants of PH and the regular invariants of H .

For example, in the Friedrichs model, H has lost the discrete eigenvalue of PH in the dissipative case. The complete set of invariants of H has been calculated and it has been verified that in the dissipative case there exists no regular invariant^{36,40}.

Although dissipativity implies the breaking of the one-to-one correspondence between the invariants of PH and of H , the inverse is not true. Poincaré's work^{9,43} provides simple examples of finite classical systems in which H admits no trivial invariants which are analytical in the coupling constant.

Nonetheless it is easy to show that in these cases the limit (26) does not exist because of the finiteness of the system. The breaking of the correspondence between the invariants of PH and of H is therefore a necessary but not a sufficient condition for dissipativity.

Causal Dynamics

The chief conclusion of my approach here is that dissipative properties correspond to a breaking of the Lt symmetry. This symmetry breaking was, however, obtained through the study of the integral representation (17) which as we have mentioned, includes causality. I shall now establish a differential equation of motion which exhibits explicitly this symmetry breaking and would therefore be the natural generalization of equation (25) valid only for simple situations (such as corresponding to weakly coupled systems). This can indeed be done in terms of a new extended formulation of transformation theory (see refs 8, 28 and 38) and, especially, ref. 44.

To summarize the basic ideas: we may ask what is the relation between the original Liouville equation (7) or its integral representation (17) and an equation such as (25) which contains both odd and even terms. The point of view as developed by our group is that both equations are exact representations of the dynamic evolution of the system. This equivalence should therefore be expressible in terms of a transformation theory which would lead from equation (7) to a new equation

$$i\partial\rho/\partial t = \Phi\rho \quad (42)$$

where the evolution operator Φ would, for dissipative systems, also contain an even part in respect of L .

In other words, one has to consider a relation of the form

$$\rho = \Lambda(L)\rho \quad (43)$$

where the transformation operator Λ depends on L . Indeed substitution of equation (43) into equation (7) yields

$$\Phi = \Lambda^{-1}L\Lambda \quad (44)$$

As both representations (7) and (42) are considered equivalent the transformation Λ should preserve average values of observables, that is, it must be such that

$$\langle O \rangle_t = \text{tr} O^+ \rho(t) = \text{tr} O^+ \rho^p(t) \quad (45)$$

with

$$O = \Lambda(-L)O \quad (46)$$

$\Lambda(-L)$ has to be used in equation (46) because the evolution operator for observables is, in the Heisenberg scheme $-L$ (and not $+L$ as in the Liouville equation). Moreover the transformation has to preserve the hermitian character of the observables and the density matrix.

An important feature of these transformations is that they are non-unitary but satisfy the condition

$$\Lambda^{-1}(L) = \Lambda^+(-L) \quad (47)$$

It is only for transformations independent of L (or even in L) that one comes back to unitary transformations, but then Φ in equation (44) could not contain both even and odd terms in L . This new class of transformations has been called "starunitary" transforms. It is easily shown that equation (47) leads to the invariance of average values as expressed in equation (45). The generator of time displacement Φ can be viewed as an extension of the collision operator $\Psi(0)$ introduced earlier.

The three basic properties of $\Psi(0)$ discussed earlier are shared by the operator Φ (refs 8, 38 and 45). We may again split Φ into an odd and an even part in respect to L inversion. Equation (42) is valid for the retarded solution. The advanced solution would satisfy an equation similar to (30) in which the sign of the even part Φ is reversed. Therefore causality is explicitly included in this formulation of dynamics. For this reason we have called equation (42) the "causal" formulation of dynamics.

As $-i\Phi$ is a seminegative operator the evolution of dissipative systems may be described in terms of a Liapounoff function. I shall indeed consider the quadratic functional in the causal representation (dropping the superscript ϕ)

$$\Omega = \text{tr } \rho^+ \rho \quad (48)$$

It is easy to show using equation (42) that

$$1/2 d\Omega/dt = -\text{tr } \rho^+ (i\Phi) \rho \leq 0 \quad (49)$$

The property (49) implies that the statistical equilibrium corresponding to a minimum of Ω has a very simple meaning in the causal formulation of dynamics: all quantum states have the same probability and random phases.

We may now introduce the quantity $\mathcal{H}$ defined through

$$\mathcal{H} - \mathcal{H}_{eq} = 1/2 \log (\text{tr } \rho^+ \rho / (\text{tr } \rho^+ \rho)_{eq}) \quad (50)$$

This corresponds then to a microscopic model of entropy given by

$$S = -k\mathcal{H} \quad (51)$$

Notice that

$$\begin{aligned} \mathcal{H} - \mathcal{H}_{eq} &\geq 0 \\ d\mathcal{H}/dt &\leq 0 \end{aligned} \quad (52)$$

$\mathcal{H}$ is an additive function for independent systems.

I cannot enter here into a detailed discussion of our quantity $\mathcal{H}$ (50) which gives, in my opinion, the general dynamic interpretation of the Second Law of Thermodynamics (16) (see I. P. and F. Henin, to be published). Briefly, however, the quantity $\mathcal{H}$ (50) corresponds to the approach of the system to microcanonical equilibrium. It can only decrease in time whatever the initial conditions (at least when some regularity conditions are satisfied³⁶). This is a basic difference with Boltzmann's definition (2) which even for dilute gases requires initial conditions compatible with the validity of the kinetic equation (1).

Irreversibility is a dynamic property based on the possibility to subdivide the solutions of the equation of motion into two semigroups corresponding respectively to retarded and advanced solutions. Entropy increases with time for each retarded solution.

Note that because the entropy is a non-linear functional of the density matrix it cannot in general be expressed in terms of observables such as temperature and density. This is only possible in the neighbourhood of equilibrium (ref. 46 and I. P. and F. Henin, to be published). It is only then that both entropy and entropy production acquire a macroscopic meaning. Briefly, the Second Law of Thermodynamics becomes a theorem in dynamics which only near equilibrium can be expressed in terms of macroscopic variables.

I shall finally compare the causal representation to the representation in which the Hamiltonian is diagonal. In such a representation one would have non-interacting excitations. Now obviously in the interaction of systems with continuous spectrum two quite different effects have to be taken into account: a modification of the interacting units ("renormalization") and an overall effect of a thermodynamic nature which expresses that the interaction permits to the system to approach equilibrium. This thermodynamic aspect of interaction has already been emphasized in the classical Einstein work on the interaction of matter with radiation⁷. The star unitarity transformation permits to treat both effects of the interaction. For non-dissipative systems one returns to the usual unitary transformation in which the only effect of the interaction terms is to redefine the excitations.

These problems will be treated more completely elsewhere (see I. P., C. George, A. Grecos and F. Henin, to be published).

Dissipative Structures

I shall now make some remarks pertaining to macroscopic thermodynamics. Once an entropy can be associated with the dynamic evolution of a system it is possible to distinguish between near and far from equilibrium situations.

This leads precisely to a distinction between "equilibrium structures"⁴⁸ which may be understood in terms of classical equilibrium thermodynamics and "dissipative structures". The latter are formed and maintained through the exchange of energy and matter in non-equilibrium conditions. Many examples of dissipative structures in fluid dynamics or non-linear chemical networks are now known. Their formation always requires a minimum distance from thermodynamic equilibrium and their appearance expresses the fact that the "thermodynamic branch", which corresponds to the extrapolation of equilibrium to non-equilibrium situations, becomes unstable against fluctuations.

Of special interest are symmetry-breaking dissipative structures in which the system has the "choice" between various possibilities. The type of initial fluctuations determines the specific dissipative structures which will be realized. Once realized this dissipative structure is stable against the type of fluctuations considered. Successions of instabilities of this type may occur, involving a kind of primitive memory or, so to say, a "historical dimension"^{48,49}. The present structure of the system can only be understood in terms of the succession of instabilities through which it originated.

There seems to be little doubt that there is a close connexion between dissipative structures and biological organization. For example, important biological multi-enzymatic processes have been shown to work beyond the stability limit of the thermodynamic branch. More details may be found in ref. 49. These considerations have to be borne in mind when the problem of the origin of irreversibility is discussed.

I shall conclude this article with some epistemological considerations about the origin of irreversibility. (For more details see refs 50-52.)

The Second Law of Thermodynamics has been repeatedly considered as the basis for the introduction of the concept of irreversibility. Others, on the other hand, base their explanations of irreversibility on biological functions such as memory. My approach leads to a model which incorporates both ideas.

To obtain the dissipativity condition (31) it was necessary to start with the formal solution (17) which as already emphasized, includes causality. Once condition (31) is satisfied, all retarded solutions lead to an increase of entropy in time. Now the incorporation of causality means that the future can at the outset be distinguished from the past. Then, using the laws of mechanics and, specifically, the dissipativity condition, one derives for such systems the existence of a thermodynamic description. In the framework of this description the concept of dissipative structures with their possible time anisotropy may be defined as in the previous section. This sequence may be expressed graphically⁵⁰⁻⁵² as in Fig. 1. Without the distinction between future and past, entropy could not be introduced, but without entropy dissipative structures in which the distinction between past and future is, so to speak, built in, could not be obtained. If we wish we may consider ourselves as a specific dissipative structure. This interpretation of irreversibility means then that we recognize in the outside world classes of objects which have precisely the property (anisotropy of time) which we have attributed to ourselves at the start.

The concept of irreversibility, and especially the Second Law of Thermodynamics, plays an important role in many parts of theoretical physics. Our unified formulation of

dynamics and thermodynamics is therefore likely to open new perspectives for theoretical research.

This article summarizes some aspects of work done by our groups in Brussels and Austin. I thank my colleagues and friends M. de Haan, Cl. George, A. Grecos, F. Henin, F. Mayné, G. Nicolis, L. Rosenfeld and G. Stey for elaboration of the ideas summarized here. This work has been supported by the Welch Foundation, Houston, and the Fonds de la Recherche Fondamentale Collective, Belgium.

- ¹ Boltzmann, L., *Wien. Ber.*, **66**, 275 (1872).
- ² Hirschfelder, J. O., Curtiss, C. F., and Bird, B. R., *Molecular Theory of Gases and Liquids* (Wiley, New York, 1951).
- ³ Alder, B. J., and Wainwright, T. E., *J. chem. Phys.*, **33**, 1439 (1960).
- ⁴ Alder, B. J., and Wainwright, T. E., *Phys. Rev.*, **127**, 359 (1962).
- ⁵ Bellemans, A., and Orban, J., *Phys. Lett.*, **24A**, 620 (1967).
- ⁶ Prigogine, I., *Acta phys. austriaca*, suppl. X, 401 (1973).
- ⁷ George, Cl., Prigogine, I., and Rosenfeld, L., *Koninkl. Dansk. Vid. Mat-Phys. Medd.*, **38**, 12 (1972).
- ⁸ Prigogine, I., George, Cl., Henin, F., and Rosenfeld, L., *Chem. Scripta*, **4**, 5 (1973).
- ⁹ Prigogine, I., *Non-Equilibrium Statistical Mechanics* (Wiley-Interscience, New York and London, 1962).
- ¹⁰ Lebowitz, J., *Proc. Sixth I.U.P.A.P. Conf. stat. Mech.*, March 1971, 41 (University of Chicago Press, 1972).
- ¹¹ Farquhar, I. E., *Ergodicity and Related Topics, Irreversibility in the Many-body Problem*, 31 (Plenum, New York and London, 1972).
- ¹² Emch, G., *Proc. Sixth I.U.P.A.P. Conf. stat. Mech.*, March 1971, 65 (University of Chicago Press, 1972).
- ¹³ Klein, G., and Prigogine, I., *Physica*, **19**, 74, 89, 1053 (1953).
- ¹⁴ Hemmer, P. G., thesis, Univ. Trondheim (1959).
- ¹⁵ Mazur, P., and Montroll, E., *J. math. Phys.*, **1**, 70 (1960).
- ¹⁶ Rubin, R. J., *J. math. Phys.*, **1**, 309 (1960).
- ¹⁷ Rubin, R. J., *J. math. Phys.*, **2**, 373 (1961) and references.
- ¹⁸ Wergeland, H., in *Irreversibility in Many-Body Problems, Sitges Int. School Phys.*, May 1972, 107 (Plenum, New York and London, 1972).
- ¹⁹ Reichenbach, M., *The Direction of Time* (University of California Press, Berkeley, and Los Angeles, 1956).
- ²⁰ Costa de Beauregard, O., in *Time in Science and Philosophy* (edit. by Zeman, J.) (Czechoslovak Academy of Sciences, Prague, 1971).
- ²¹ Baus, M., *Acad. r. Belg. Bull. Cl. Sci.*, **53**, 1291, 1332, 1352 (1967).
- ²² Lanz, L., and Lugato, L. A., *Physica*, **44**, 532 (1969).
- ²³ Grecos, A., *Physica*, **51**, 50 (1970).
- ²⁴ Résibois, P., and De Leener, M., *Phys. Rev.*, **178**, 806, 819 (1969).
- ²⁵ Résibois, P., Piasecki, J., and Pomeau, Y., *Phys. Rev. Lett.*, **28**, 882 (1972).
- ²⁶ Mayné, F., and Prigogine, I., *Physica*, **63**, 1 (1973).
- ²⁷ Baus, M., thesis, Free University of Brussels (1968).
- ²⁸ Henin, F., and De Haan, M., *Physica* (in the press).
- ²⁹ Stey, G., *Physica* (in the press).
- ³⁰ Gibberd, R. W., and Stey, G., *Physica*, **62**, 301 (1972).
- ³¹ Prigogine, I., and Severne, G., *Physica*, **32**, 1376 (1966).
- ³² Severne, G., *Physica*, **61**, 307 (1972).
- ³³ Glazman, I., and Liubitch, Y., *Analyse linéaire dans les espaces de dimensions finies*, 352 (Ed. Mir, USSR, 1972).
- ³⁴ Grecos, A., *Proc. natn. Acad. Sci. U.S.A.* (in the press).
- ³⁵ Friedrichs, K., *Comm. Pure appl. Math.*, **1**, 361 (1948).
- ³⁶ Grecos, A., and Prigogine, I., *Physica*, **59**, 77 (1972).
- ³⁷ Grecos, A., and Prigogine, I., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1969 (1972).
- ³⁸ De Haan, M., *Physica* (in the press).
- ³⁹ Bongaerts, P. J. M., Fannes, M., and Verbeure, A. (preprint).
- ⁴⁰ Prigogine, I., and Grecos, A., *Volume in Honour of H. Frohlich* (edit. by Haken, H.) (in the press).
- ⁴¹ Balescu, R., Clavin, P., Mandel, P., and Turner, J. W., *Acad. r. Belg. Bull. Cl. Sci.*, **55**, 1055 (1969).
- ⁴² Shimizu, T., *Prog. theor. Phys.*, **47**, 1181 (1972).
- ⁴³ Poincaré, H., *Méthodes Nouvelles de la Mécanique Céleste*, Gauthier-Villars (1892, reprinted by Dover, New York, 1957).
- ⁴⁴ George, Cl., *Physica*, **65**, 277 (1973).
- ⁴⁵ De Haan, M., thesis, Free University of Brussels (1973).
- ⁴⁶ Prigogine, I., in *Foundations of Continuum Thermodynamics* (edit. by Domingos) (in the press).
- ⁴⁷ Einstein, A., *Physik Z.*, **18**, 121 (1917).
- ⁴⁸ Glansdorff, P., and Prigogine, I., *Stability, Structure and Fluctuations* (Wiley-Interscience, 1971; French edition, Masson, Paris, 1971).
- ⁴⁹ Prigogine, I., Nicolis, G., and Babloyantz, A., *Phys. Today* (November and December, 1972).
- ⁵⁰ Prigogine, I., *Acad. r. Belg. Bull. Cl. Sci.*, **58**, 1189 (1973).
- ⁵¹ Prigogine, I., *Proc. Symp. Development of the Physicist's Conception of Nature, Trieste, Italy, 1972* (edit. by Mehra, J.) (Reidel, Dordrecht, and Boston, 1973).
- ⁵² Prigogine, I., in *Connaissance Scientifique et Philosophie, Colloque du Bicentenaire de l'Académie Royale de Belgique* (1973) (in the press).

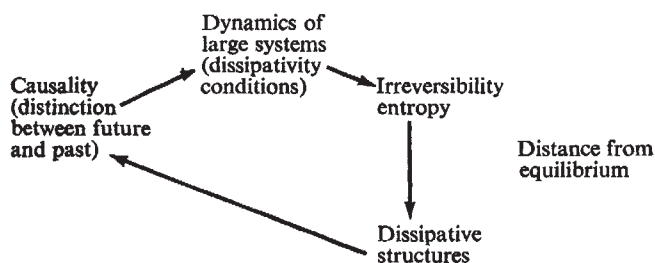


Fig. 1

Eclipse Flight of Concorde 001

J. BECKMAN

Queen Mary College, Mile End Road, London

J. BEGOT

Institut d'Astrophysique, 98 bis Boulevard Arago, 75014 Paris

P. CHARVIN

Institut National Astronomie et Geophysique, Place J. Janssen, 92190 Meudon

D. HALL

Kitt Peak National Observatory, PO Box 4130, Tucson, Arizona

P. LENA &

A. SOUFFLOT

Université Paris VII, Observatoire de Meudon, 92190 Meudon

D. LIEBENBERG

Los Alamos Scientific Laboratories, Los Alamos, New Mexico

P. WRAIGHT

University of Aberdeen

On June 30, 1973, Concorde 001 intercepted the path of a solar eclipse over North Africa. Flying at Mach 2.05 the aircraft provided seven observers from France, Britain and the United States with 74 min of totality bounded by extended second (7 min) and third (12 min) contacts. The former permitted searches for time variations of much longer period than previously possible and the latter provided an opportunity for chromospheric observations of improved height resolution. The altitude, which varied between 16,200 and 17,700 m, freed the observations from the usual weather problems and greatly reduced atmospheric absorption and sky noise in regions of the infrared.

THE Concorde took off from Las Palmas in the Canaries, intercepted the eclipse over Mauritania, paced the umbra along a great circle path over Mali, Algeria, Niger and part of Tchad, and landed in Fort Lamy. The astronomical requirement of stable flight throughout both a maximum period of totality and the second and third contacts (natural contacts) imposed a rigid schedule on the Concorde. Had the aircraft been 2 min early on arrival in the eclipse path, the period of totality would have been reduced by 25 min and one natural contact would have been lost. On the day of the eclipse the Concorde took off and intercepted the umbra within 5 s of the allotted times after 45 min of flight; it maintained maximum ground speed (544 m s^{-1}) (Mach 2.00 to 2.10) throughout the observations and the duration of totality was determined by meteorological conditions in the stratosphere. (We note that the exact definition of second and third contact may have to be re-examined when

the transit of the Moon is very slow.) There was no interference with ground sites and observers. All this is a magnificent achievement on which we congratulate Captain Turcat and his crew.



Fig. 1 A view of the aircraft, showing the four special purpose windows on the top. From front to back: crystal quartz window, composite Irtran-2 window, two fused quartz windows. (Picture by Aérospatiale.)

The Concorde carried equipment for experiments by five groups. The instruments and their performance are discussed in the following sections. Apart from four window assemblies, which were mounted in the roof (see Fig. 1), the aircraft required no structural modifications as the equipment was attached to the standard floor tracks. The limitations on space and weight were not severe. The Concorde was made available for five test flights before the eclipse including three Sun-tracking flights. During the eclipse itself the stability of the aircraft was excellent: pitch $\lesssim 0.1^\circ$, roll $\lesssim 0.5^\circ$ (peak-to-peak values). An unusual

problem was that, during the eclipse, the attitude of the Sun varied from 27° below zenith ahead of the aircraft to nearly overhead and then to 23° below zenith behind the aircraft.

Here we describe the five experiments, going from the front to the back of the aircraft's cabin.

Far Infrared Emission from the Chromosphere

The purpose of this experiment (Queen Mary College, London) was to examine the upper chromosphere using its emission between $300\ \mu\text{m}$ and $1,500\ \mu\text{m}$. An eclipse gives a necessary condition for this observation, for only lunar occultation of successive layers can produce the required angular resolutions, of order 1 arc s. The best that can be done outside eclipse is around 40 arc s using a large telescope such as the 36-foot NRAO dish at Kitt Peak, because of diffraction limitations. Further, the effective slowing down of the Moon's passage by a factor of ten, enables a high enough signal to noise ratio to be obtained to use the angular resolution available. A final advantage of using the Concorde was that at heights of over 50,000 foot, well above the tropopause, radiation between $300\ \mu\text{m}$ and $1,000\ \mu\text{m}$ reaches the equipment almost unaffected by absorption due to the water vapour rotational spectrum. At ground sites, the average transmission in this wavelength range is only a few per cent.

Our equipment comprises a Rollin InSb photoconductive detector, cooled to liquid He temperature, with useful detectivity between $250\ \mu\text{m}$ and 2 mm. This was fed by a conventional Michelson spectral interferometer, having a $50\ \mu\text{m}$ thick melinex beamsplitter, and 6-cm diameter plane reflectors. The interferometer could be switched to scan through a path difference of 2 cm in 1 s, giving audio output frequencies between 10 Hz and 80 Hz, or ten times more slowly. In the former case analogue Fourier transformation could be performed with suitable electrical filter networks; in the latter digital transformation is needed, to obtain the spectrum of the radiation. The interferometer was fed by an image of the whole eclipsed disk, through a chopper at 1 kHz, used in conjunction with a phase sensitive detector at the output of the radiation detector. Optically the system was very simple: the solar beam passed through the aircraft window, a disk of single crystal quartz, 12 cm in diameter chosen for its transmission properties. It was folded with several plane mirrors on to a lens of TPX plastic, also of high far infrared transmission, into the interferometer. One of the plane mirrors had to be moved electrically at a pre-calculated nearly linear rate along the aircraft axis during totality. A second was guided about the roll axis electrically, using a servo circuit linked to the inertial navigation system of the aircraft, and about the pitch axis manually. A small optical telescope aided the guiding, fed by a 2-cm flat, through which the observer could see the eclipse and surrounding coronal light. Invaluable help was given in designing and constructing the equipment by the National Physical Laboratory Electrical Science division, by the Science Research Council's Culham Laboratory, and particularly by the Radio and Space Research Station, Slough.

The experiment can be counted a success, in that all the individual systems worked, and data were obtained on chart and magnetic tape. Preliminary analysis of the data is consistent with emission detected from the chromosphere, of the right intensity compared with the lunar far infrared signal, and covering the correct angular range outside the lunar disk. The chromospheric signal seems to have been detected not only during the 7 min before photospheric second contact (onset of totality) and 12 min after third contact, but for some 5 min after second and before third contacts.

Infrared Emission of the Thermal Corona

The aim of this experiment (Observatoire de Meudon) was to assess the emission of the thermal corona, or dust corona, in the wavelength range 2 to $13\ \mu\text{m}$. The main advantages of mounting it onboard Concorde are: (i) better atmospheric transmission at 17,000 m; (ii) correspondingly reduced fluctuations of the atmospheric emission which seem to have hampered ground observations at the 1970 Mexican eclipse; (iii) longer integration time.

The window assembly consisted of a circle of four Irtran-2 disks, each of clear diameter 10 cm, with a 3-cm fused quartz window at the centre for guiding purposes. All the optics were mounted on a sliding table, shock-mounted to the floor, to follow, without vignetting, the apparent motion of the Sun in the aircraft. A gyro-heliostat, provided by NASA, stabilised the image on an off-axis, 50 cm diameter, $f/3$ telescope. Image acquisition and scanning were achieved manually by visual control of the image in the focal plane. Differential modulation in this plane switched two diaphragms, 4.4 arc min in diameter, 22 arc min apart in order to balance instrument emission. The beam was divided into three measurement channels, covering respectively 2 to $3\ \mu\text{m}$, 4 to $5\ \mu\text{m}$, 8 to $13\ \mu\text{m}$. The detectors were respectively liquid N_2 cooled InSb photoconductors and liquid He cooled Ge-bolometer.

All three channels could be operated either wide band, or with spectral resolution of about 50 provided by a grating or by a filter wheel (8 to $13\ \mu\text{m}$ channel). Data, including various parameters on board, were recorded both in analogue and in digital form. Total weight of the experiment was about 400 kg.

All the subsystems performed properly during the flight, and the Moon+corona acquisition proved easy. Tracking stability stayed better than 1 arc min for most of the flight. Fifty-six minutes were devoted to wide band scans along and perpendicular to the ecliptic, from 3 to 8 solar radii. Eighteen minutes were devoted to spectral scans at 3.9 solar radii (the area of maximum emission at $10\ \mu\text{m}$).

The radial scans should allow confirmation of previous results at shorter wavelengths, to correlate the longer wavelength emission with them, and hopefully to throw some light on particles material identification through their emission spectra in the $10\ \mu\text{m}$ band.

Invaluable help in building the equipment was provided by the Laboratoire de Physique Stellaire et Planétaire and the Services des Prototypes, both CNRS Institutes, by the Centre National d'Etude des Télécommunications and by Kitt Peak National Observatory.

Photographic Study of the Corona

The experiment (Institut d'Astrophysique de CNRS, Paris) aimed to measure the lifetime of typical structures in the inner corona ($r < 1.5 R_\odot$), to search for oscillatory motions, and to study the outer corona ($r < 15 R_\odot$), by photographic photometry. Three cameras were mounted on a manually oriented gimbal, observing through a fused quartz window 135 mm in diameter. Respective focal lengths are: 180 mm, 1,520 mm, 3,000 mm. Kodak Recording 247S, TRI-X-P and Ektachrome EF films were used. Results on the three systems are as follows.

(1) Several photographs at wide field exposed at 1/30 and 1/60 s; sky brightness appears very low at 17,000 m and should allow a study of the corona at large distances.

(2) Thirty-seven photographs $60 \times 60\ \text{mm}$, 2 min apart, on the 3 m camera, 1/60 s exposure; resolution is of the order of 2 arc s. (Some of these are shown in Fig. 2.)

(3) Two hundred and thirty colour photographs (24×36 format), on the 1.5 m camera. The black and white photographs are calibrated.

Structural changes in the corona are noticeable at the poles, as well as in a coronal enhancement on the eastern side. Photometric study of the films should allow a determination of the lifetime of the polar plumes and an investigation of relaxation processes in the inner corona. Propagation of a coronal wave at the W limb and at about $1 R_{\odot}$ from the limb may be studied. The high spatial resolution should allow investigation of the effect of a supersonic shock wave on the wave front, and thus on the image quality.

High Resolution of the Emission Line Corona

These observations (University of California, Los Alamos Scientific Laboratory, Los Alamos) had two purposes; (1) to determine whether the mechanical periodicities of 5 min observed in the photosphere contribute to the heating of the corona; (2) to use the slow motion shuttering of the solar atmosphere by the lunar limb to observe the development of the emission corona just above the chromosphere.

If the corona-chromosphere transition is about 1,000 km thick, this latter observation would be completed in about 3 s for an observer at a ground site while on Concorde about 30 s were available.

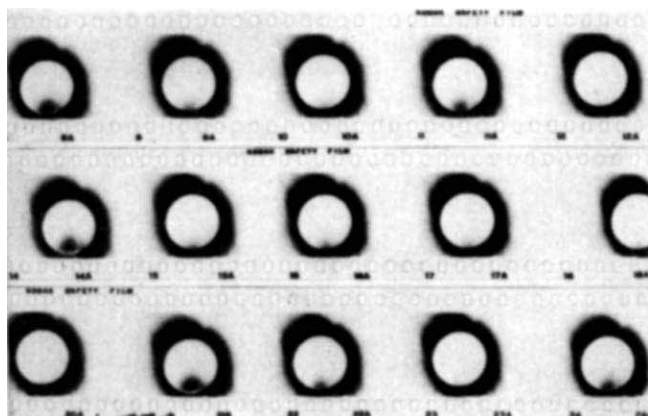


Fig. 2 Successive photographs of the solar corona, taken with $f=3$ m every 20 s, exposure 1/60 s. (J. Begot, Institut d'Astrophysique.)

A single 10-cm fused silica window was left uncoated because of the large altitude change of the Sun during totality. Close to this window a two-axis mirror was located to direct the light into a telescope of aperture 7.6 cm and focal length 1 m. The mirror was guided from photoelectric error signals produced on a tracking focal plane by splitting off 10% of the light from the main beam. The long focal length and consequent large f number was used to obtain a good image scale so that resolution to 10 arc s was available. The main beam was recollimated and passed through an interference filter with a square pass band about 9 Å full width at half intensity points. Then spectral scanning through the $\lambda 5303$ emission line was accomplished with a pressure scanned Fabry-Perot interferometer. The image was reformed without change in magnification onto the image intensifier surface optically linked to the vidicon tube. A video frame was obtained every 20 ms and the interferometer finesse, about 50, and scan rate, were adjusted to give 0.5 mm (0.05 Å) resolution. Digitisation and subsequent analysis as previously used will be accomplished at Los Alamos. This detailed spectral study was complementary to the successful observations in other coronal emission lines carried out by

other Los Alamos scientists on the NC-135 that was passed by the Concorde near the mid-eclipse point. The instrument weighed about 250 kg.

The main objectives of the observations were achieved. Good spectral line profiles were obtained, and the relative intensity calibration on the video recording will give reliable detection of periodic intensity changes. Although some tracking difficulties were encountered with the photoelectric tracker during the eclipse, adequate tracking was obtained during totality and around third contact to give emission line profiles for both the above purposes.

Particularly striking to the observer was the very dark sky on which a brilliant blue white corona was superposed like a cameo. Near third contact the emergence of the red (hydrogen line-emitting) chromosphere was particularly beautiful as the chromospheric crescent protruded from behind the lunar limb and spread around about a quarter of the solar circumference before the photosphere emerged to end totality. Of course the corona could be seen well after third contact and up to the time we turned away to land at Ft Lamy.

Near Infrared Emission of the Upper Atmosphere

A two-channel spectrophotometer (University of Aberdeen) was used to study the infrared atmospheric system of molecular oxygen in emission. Interference filters with peak transmissions close to $1.27 \mu\text{m}$ and $1.58 \mu\text{m}$ were tilted by motor-driven cams to give spectral scans approximately $0.04 \mu\text{m}$ in range every 60 s. The widths of the passbands of the filter were $0.007 \mu\text{m}$ and $0.01 \mu\text{m}$ respectively. The spectrophotometer was placed close to a passenger window towards the rear of the fuselage in a mounting that permitted selection of the elevation angle of the field of view between 0° (horizontal) and 40° .

The regular dayglow at $1.27 \mu\text{m}$ and $1.58 \mu\text{m}$ was observed during all flights and good measurements of the $1.27 \mu\text{m}$ emission were obtained throughout the total eclipse. The $1.58 \mu\text{m}$ emission disappeared into noise before third contact but was seen clearly before second contact. Observations were obtained at a number of differing elevation angles during totality as well as during the test flights and it is clear that the data will yield estimates of (a) the intensities of the two emission radiances and their ratio; (b) the effective atmospheric absorption of the $1.27 \mu\text{m}$ emission; (c) the effective quenching rate for the $^1\Delta_g$ excited level of O_2 ; (d) the variation with latitude of the $1.27 \mu\text{m}$ emission radiance; and (e) the lack of medium-scale irregularities in the dayglow radiance.

The full results of the individual experiments will be published by the various participating groups; but it is already worth pointing out how profound a change in eclipse observations has accompanied the advent of supersonic aircraft, which has proved to be a very successful carrier in this particular project. There can be no doubt that future plans should favour this method over more conventional approaches.

The project was made possible through the French Délégation Générale à la Recherche Scientifique et Technique and was coordinated by the Institut National d'Astronomie et de Géophysique of the Centre Nationale de la Recherche Scientifique with the full support of the Aérospatiale Company. French experiments were supported by the Institut National d'Astronomie et de Géophysique, British experiments were supported by the Science Research Council and the United States experiment by the National Science Foundation.

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Geomorphological Dating of Cave Openings at Makapansgat, Sterkfontein, Swartkrans and Taung

T. C. PARTRIDGE

PO Box 31023, Braamfontein, Transvaal

Cave opening at several of the South African hominid sites occurred through incision and valley widening in one current erosion cycle. Measurements of rates of cyclic nickpoint migration and valley flank recession make possible time-place transpositions, and the approximate date of cavern opening can be determined for each site. In each case this date represents a *terminus post quem* for the accumulation of hominid-bearing deposits.

ABSOLUTE dating of the early hominid cave deposits in South Africa has long been of concern to anthropologists, because unresolved problems of taxonomy and phylogeny stem largely from a lack of comparative chronological data for these sites. In particular, the chronological position of *Australopithecus africanus* remains in doubt. Faunal assemblages from the South African sites are often too restricted in their range of species to permit correlations either between sites, or with dated assemblages from East Africa.

Although radioisotopic and other techniques have been applied to the South African sites no absolute dates have been obtained. I therefore set out to investigate the possibility of applying geomorphological techniques of age determination in the hope that these might throw light on the date of accumulation of the South African cavern deposits.

Erosion Cycles

The method is based on a quantitative assessment of rates of cyclic erosion relative to well-established geomorphological datum levels. Since the Jurassic the African continent has been subject to five active episodes of epeirogenic movement, separated by intermissions in which erosion has advanced inland from the coast, with concomitant sedimentation of the continental shelf. The ages of these movements and the resulting offshore deposition are well known on the basis of both tectonic and marine palaeontological studies¹⁻⁵.

The recurrent pattern of continental landform development which succeeded each active episode has been the subject of detailed analysis^{6,7}; it is significant that recession of valley nickpoints inland from the coastal margin was

followed by valley widening, scarp retreat and pedimentation, leading ultimately to the formation of gently undulating pediplains or erosion surfaces. Where such surfaces have reached an advanced stage of planation local relief is minimal, and their distribution may be mapped very accurately. In some areas such as the South African Highveld, these plains extend unbroken for thousands of square km, and transgress both structure and lithology without interruption. Their existence in the African landscape is irrefutable, and their distribution has been described and mapped by several workers^{2-4,7-12}.

The erosion cycles seen near the South African hominid deposits are the African and Post African I. The most recent (Plio-Pleistocene) cycle is represented over much of South Africa by gorges incised into the coastal hinterland, and advanced planation referable to it is restricted to local basins. The date of inception of these cycles, based on palaeontological studies of the equivalent marine sediments, is as follows:

Table 1 Erosion Cycles in South Africa*

Cycle	Marine depositional equivalent	Date of inception
African	Littoral Senonian strata of east coast, with succeeding Eocene in Mocambique; Late Cretaceous and Eocene strata of Angola	Mid-Cretaceous (100 m.y. BP)
Post African I	Burdigalian marine series	Miocene (20 m.y. BP)
Plio-Pleistocene	Red "Berea" and other coastal sands	(2 m.y. BP)

* Adapted from King³.

The African erosion surface is characteristically well planed and provides a readily identifiable datum level in all areas unconsumed by later incision; however, local deformations along well defined axes are present, and are related to the tectonic disturbances which initiated the Plio-Pleistocene incision¹³. One such axis coincides with the Continental divide along the Witwatersrand ridge some 30 km to the south of the Sterkfontein and Swartkrans sites, and has resulted in upwarping of the African surface evident in the marked northerly tilt of discontinuous benches eroded in this cycle along the upper flanks of the Bloubaank Valley. Here African benches decline in elevation downstream at approximately 6 m km⁻¹.

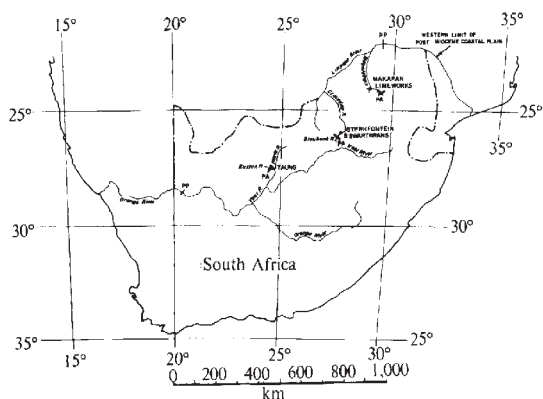


Fig. 1 Map of South Africa showing hominid sites, drainage systems and nickpoints referred to in text. ●, Hominid sites; PP |, Plio-Pleistocene nickpoint; PA ||, Post-African nickpoint

Nickpoint Migration

Figure 1 shows the locations of the sites and drainage systems which I discuss. The positions of the African, Post-African I and Plio-Pleistocene nickpoints, which represent the furthest point reached by incision in each cycle, are indicated relative to the streams concerned. In every case the hominid site is situated in a valley incised below the African erosion surface in the Post-African erosion cycle; valley or cave opening therefore occurred under the influence of this incision.

Figure 2 provides a graphical representation of the rate of nickpoint migration inland from the coast expressed as a proportion of the total stream length. The rate of nickpoint migration has been calculated by measuring the length of the stream channel segments affected by the Plio-Pleistocene and Post-African I incision. The mean rate of nickpoint migration was then plotted in relation to that proportion of the channel length represented by the mid-point of each segment, taking into account the position of the coast at the time of inception of the cycle which produced the nickpoint concerned. In the absence of supplementary evidence, it was assumed that the nickpoint migration rate between each of these points was subject to a linear decline culminating in zero at the point of origin of the stream in the watershed area.

This assumption is, of course, erroneous in that nickpoint migration rates are undoubtedly influenced by variations in lithology, climate, tributary flow and sediment load. Most of these influences are not, however, susceptible to quantitative assessment, although reference to Table 2 shows that the lithological composition of the valleys is comparable in each basin. Hence lithological modifications of the linear relationship are likely to be generally equivalent in each case. Moreover the errors in the use of the linear interpolation technique are likely to be reduced greatly by the close proximity of each site to the headwater of the local stream (see Fig. 1). In no instance does this exceed 14.24 km or 2.71% of the channel length, and migration rates so near to the stream source are so low, owing to the small available erosive potential, as to be little affected by possible departures from the linear mode of decline.

The nickpoint migration graphs presented in Fig. 1 demand some comment. Reference should be made to Table 3 in which the measurements from which the graphs have been constructed are presented. The graphs are numbered 1, 2 and 3 to indicate the drainage systems concerned: (1) the Limpopo-Magalakwin-Makapan drainage system, containing the Makapansgat limeworks site (a); (2) the Limpopo-Crocodile-Bloubank drainage system, containing the Sterkfontein and Swartkrans sites (b); (3) the Orange-Vaal-Harts-Buxton drainage system, containing Taung site (c) at the Buxton lime quarry.

Table 2 Occurrence of Various Lithological Types in Drainage Basins

Rock types	% of total channel length in drainage basins		
	1*	2*	3*
Moderately resistant Palaeozoic and Mesozoic sediments	30.25	29.43	27.06
Resistant Precambrian basic igneous rocks, largely plutonic	2.52	3.65	1.65
Resistant Precambrian acid igneous rocks and gneisses, largely plutonic	38.23	44.00	36.93
Very resistant Precambrian sediments and meta-sediments	29.00	22.92	34.36

* Drainage basins: 1, Limpopo-Magalakwin-Makapan; 2, Limpopo-Crocodile-Bloubank; 3, Orange-Vaal-Harts-Buxton.

The lower reaches of drainage systems (a) and (b), below the Magalakwin-Limpopo confluence, are common in the lower course of the Limpopo river, which enters the Mocambique coastal plain just to the east of the South African border. This plain is largely underlain by marine sediments, and was elevated to its present position by post-Miocene movements⁶. For this reason the segment of the Limpopo channel crossing it was ignored in measuring the extent of Post-African I nickpoint recession from the coast.

All three graphs are of similar form with a conspicuous inflection indicating substantially different rates of nickpoint

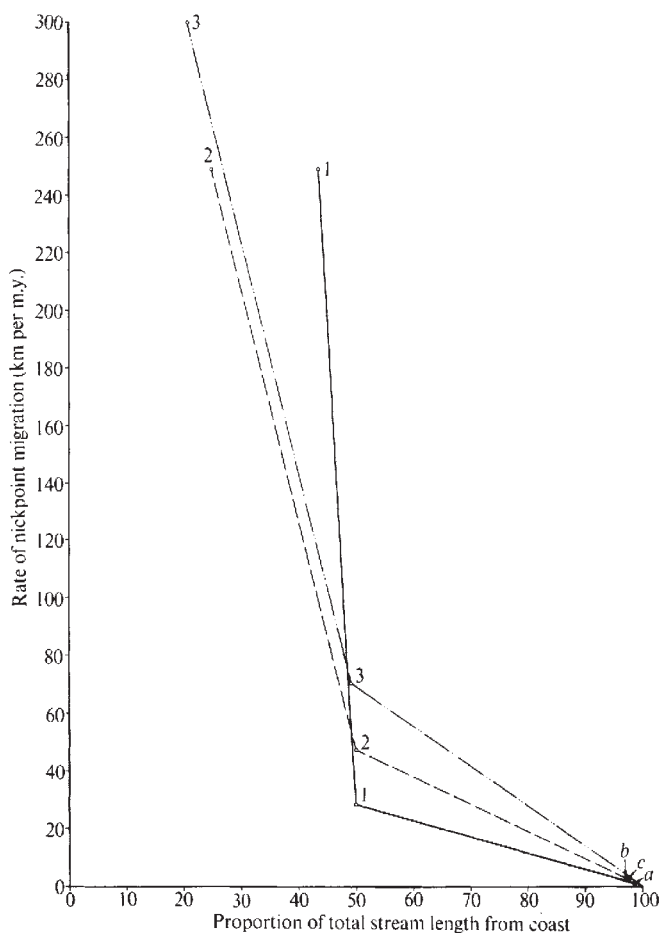


Fig. 2 Rate of nickpoint migration inland from coast. Sites, a, Makapansgat limeworks; b, Sterkfontein and Swartkrans; and c, Taung. Drainage systems (1) Limpopo-Magalakwin-Makapan; (2) Limpopo-Crocodile-Bloubank; and (3) Orange-Vaal-Harts-Buxton.

migration in the Post-African I and Plio-Pleistocene erosion cycles. The more rapid rate of recession in the Plio-Pleistocene cycle is undoubtedly the result of the greater magnitude of the associated uplift and warping of the continental margin. Variations in rates of nickpoint migration between the different drainage systems may be explained by differences in the degree of stream activity, as dictated by volume and regularity of flow. Hence the lowest rates are evident in the Limpopo-Magalakwin-Makapan system which is non-perennial over much of its length, and the highest rates occur in the Orange-Vaal-Harts-Buxton system, which is subject to a more regular flow, particularly below the Vaal-Harts confluence.

From these measurements it is possible to determine the mean rate of nickpoint migration between each site and the current position of the Post-African I nickpoint, which is situated in each case a short distance upstream. In all drainage basins this channel segment is contained almost entirely within the lower part of the Dolomite Series, Transvaal System, so that the calculated migration rates should not involve errors caused by major lithological variations. Division of this distance by the mean rate of nickpoint migration associated with it, gives the date before present of the arrival of the Post-African I nickpoint opposite each site. These dates are given in Table 3. They merely provide, however, a *terminus post quem* for the valley deepening and widening during which cavern opening occurred, or the locale was created for the accumulation of the hominid-bearing deposit. In order to determine the further time lapse required for this to occur it is necessary to consider briefly the geomorphological environment of each of the sites.

Analysis of Cave Sites

All of the deposits occur at similar stratigraphic levels within the Dolomite Series, which consists of interbedded

horizons of soluble dolomite and insoluble chert. This rock is subject to cycles of solution and cavern development closely linked to the cycles of subaerial erosion described previously^{10,11}. After the solutional formation of a cavity beneath the water table, incision and reduction of local base level produced lowering of the phreatic surface; concomitant valley widening caused the land surface to intersect the cavity creating an opening to the surface, commonly through a vertically enlarged joint or aven. This opening permitted the ingress of externally derived hillside soil, as well as hominids and animals. Hence, to determine the date of the earliest accumulation of the cave deposit, it is necessary to determine the time period which elapsed between arrival of the new erosion cycle opposite the site and recession of the valley side to a point at which cavern opening occurred.

The situation described above pertains in the case of the Makapansgat Limeworks, Sterkfontein and Swartkrans deposits, but the Taung deposit accumulated in cavities developed in a series of calcareous tufas deposited on the valley flank at Buxton subsequent to the incision of the valley in the Post-African I erosion cycle¹⁴. Hence the geomorphological controls governing cavern opening do not apply in this case, and all that may be deduced is that the hominid-bearing deposit post-dated the arrival of the Post-African I nickpoint (0.87 m.y. BP).

In the case of the dolomite cavern deposits, the time lapse involved in the widening of the valley to produce cavern opening has been calculated as a proportion of the total valley widening in the Post-African I cycle that has occurred to date. Figures 3 and 4 show topographic sections through the Makapan and Bloubaank valleys at the limeworks, Sterkfontein and Swartkrans site, together with the local elevations of the African erosion surface. These elevations were determined through the construction and superimposition of numerous transverse and longitudinal profiles over large areas in the vicinity of each site. Both valleys are composite, in that they have been incised in both the

Table 3 Nickpoint Recession

Drainage system	Total trunk channel length (km)	Headward recession from coast (km)	recession of cyclic nickpoints % of total channel length (km per m.y.)	Rate of recession (km per m.y.)	Distance of site from coast as % of channel length	Distance of oldest upstream nickpoint from coast as % of channel length	Mean rate of recession between site and oldest upstream nickpoint (km per m.y.)	Distance between site and oldest upstream nickpoint (km)	Date in m.y. BP of arrival of oldest nickpoint opposite site	Proportion of total valley down-cutting and widening (%) in locally current erosion cycle required to open cave	Date in m.y. BP of cave opening
Limpopo-Magalakwin-Makapan	570.15	Plio-Pleistocene: 497.80	87.30	248.90	Makapansgat limeworks: 98.78	99.28	0.55	2.86	5.24	30.00	3.67
		Post African I: African: 566.21	99.28	28.36							
Limpopo-Crocodile-Bloubank	960.77	Plio-Pleistocene: 497.80	51.81	248.90	Sterkfontein: 97.29	98.77	1.85	14.24	7.71	57.77	3.26
		Post African I: 949.08	98.77	47.45	Swartkrans: 97.40		1.79	13.12	7.33	64.91	2.57
Orange-Vaal-Harts-Buxton	1,436.46	Plio-Pleistocene: 596.90	41.58	298.45	Taung: 97.94	98.11	2.73	2.38	0.87	—	<0.87
		Post African I: 1,408.83	98.11	70.45							

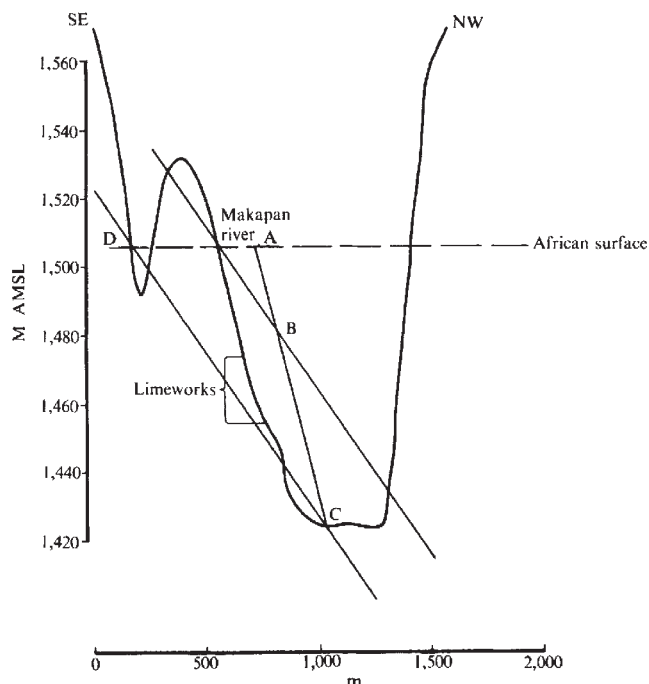


Fig. 3 Section through Makapan Valley at limeworks (see text for explanation).

African and Post-African I erosion cycles, but the presence of benches, whose projected elevations accord with one another and with nearby areas of African planation, leave no doubt as to the portion of the incision referable to the Post-African I cycle.

To determine the approximate time required for valley widening to open the caverns, a line was drawn between the base of the present stream channel and the point of intersection of the African surface with the present valley side (line CD). A parallel line was drawn through the projected apex of the aven through which cavern opening occurred, and another was inserted to indicate the downward path of the stream channel during its incision from the African level to its present position (line ABC). It should be noted that these latter lines are not vertical, but assume varying inclinations in the three sections. This reflects lateral channel migration during the course of incision, partly in conformity with the local dip of the dolomite strata, partly as a result of asymmetrical downcutting in relation to the slip-off and undercut slopes of incising meanders. The original position of the channel at the level of the African surface was carefully determined in each case from distributions of benches and terraces occurring at this and subsequent levels.

The intercept AB is a measure of the amount of incision and valley widening required to open the cavern. This, expressed as a proportion of the total incision along the downward path of the channel (AC), represents that proportion of the time which elapsed subsequent to the arrival of the Post-African I nickpoint opposite the site, required to achieve cavern opening.

In this analysis of valley widening several assumptions have been made: first, that the valley flanks approximated to a rectilinear configuration during recession; second, that the downward path of the channel during incision was rectilinear; and third, that both channel incision and concomitant valley flank recession occurred at a regular rate. None of these assumptions is likely to be wholly accurate. The probable departures from these ideal conditions are, however, unlikely to have been sufficiently great to affect the relative dates or the general order of ages indicated in the

results, particularly because incision and valley widening occurred in very similar sedimentary successions at each site.

The proportions of valley flank recession required to open each cavern are presented in Table 3, and the resulting date for the first opening of each to permit the ingress of hillside materials, hominids and animals is given. These are approximately 3.7 m.y. for the Makapansgat limeworks, 3.3 m.y. for Sterkfontein, 2.6 m.y. for Swartkrans, and, as has been indicated previously, somewhat less than 0.87 m.y. for Taung.

Implications

These ages for the first opening of caverns or locales for the accumulation of hominid-bearing deposits embody certain errors inherent in the assumptions made during their derivation. It is, however, considered unlikely that they are large enough to affect the relative dates or general order of ages that have been deduced for two reasons: first, the lithological compositions of the channels are generally similar in each basin, and almost identical between each site and its upstream nickpoint; hence possible error factors caused by lack of lithological homogeneity are likely to be fairly evenly spread relative to the various sites. Second, interpolations for valley downcutting and widening at each site have been carried out relative to the actual profile of the valley section; hence local erosional irregularities have been considered.

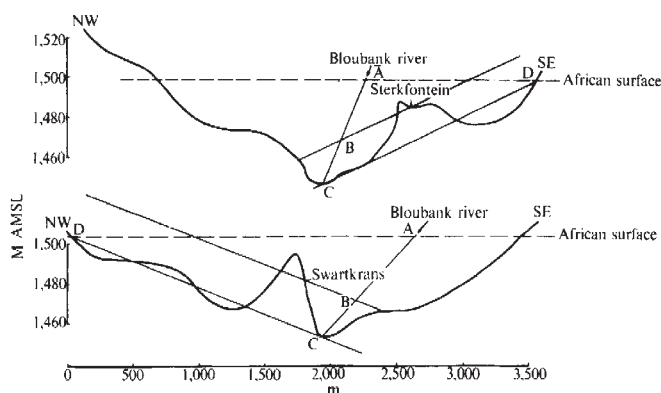


Fig. 4 Sections through Bloubank Valley at Sterkfontein and Swartkrans (see text for explanation).

Comparisons of faunal material from all sites, and the remains of *Australopithecus robustus* from Swartkrans, with similar dated material from East Africa, have suggested that the deposits at Makapansgat limeworks, Sterkfontein and Swartkrans may be at least 0.5 m.y. younger than is indicated by the dates presented here^{15,16}. It should, however, be borne in mind that these dates are for the earliest cave opening, and that strata may be present in the lower levels of the deposits which are older than those from which the comparative material was derived. Such early strata of substantial thickness have recently been recorded both at Makapansgat and Sterkfontein. Furthermore, as Butzer¹⁷ has pointed out, the local production of hillside soil which forms the bulk of the breccias is extremely slow, and tens or even hundreds of thousands of years may have been required for such extensive deposits to accumulate. The young date for the Taung site is somewhat surprising, although Wells¹⁸ has suggested, on faunal grounds, that the accumulation of the Taung hominid deposit may have occurred later than was previously thought.

I hope that, in the absence of suitable isotopic dating methods, these results may provide less precise interim data until other chronometric techniques are developed.

I thank Professor P. V. Tobias for comment and encour-

agement. This article was adapted from the Chairman's address to Witwatersrand Centre, South African Archaeological Society, delivered February 28, 1973.

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- ¹ Jessen, O., *Reisen und Forschungen in Angola* (Verlag von Dietrich Reimer, Berlin, 1936).
- ² Dixey, F., *Trans. geol. Soc. S. Afr.*, **41**, 113 (1938).
- ³ King, L. C., *The Morphology of the Earth* (Oliver and Boyd, Edinburgh and London, 1962).
- ⁴ King, L. C., *The Natal Monocline* (University of Natal, Durban, 1972).
- ⁵ King, L. C., *Z. Geomorph. N.F.*, **16**, 239 (1972).
- ⁶ King, L. C., *South African Scenery* (Oliver and Boyd, Edinburgh and London, 1967).

- ⁷ Partridge, T. C., *Some Geomorphic Units in the Transvaal and their Significance in Physical Development* (thesis, University of Natal, Durban, 1969).
- ⁸ Dixey, F., *Trans. geol. Soc. S. Afr.*, **45**, 151 (1942).
- ⁹ Dixey, F., *Trans. geol. Soc. S. Afr.*, **58** (Annexure) (1956).
- ¹⁰ Brink, A. B. A., and Partridge, T. C., *S. Afr. geogr. J.*, **47**, 11 (1965).
- ¹¹ Marker, M. E., and Moon, B. P., *S. Afr. geogr. J.*, **51**, 106 (1969).
- ¹² Doornkamp, J. C., *Geogr. Annl.*, **50A**, 151 (1968).
- ¹³ Du Toit, A. L., *S. Afr. geogr. J.*, **16**, 3 (1933).
- ¹⁴ Peabody, F. E., *Bull. geol. Soc. Am.*, **65**, 671 (1954).
- ¹⁵ Tobias, P. V., in *The Functional and Evolutionary Biology of Primates* (Aldine-Atherton, Chicago and New York, 1972).
- ¹⁶ Cooke, H. B. S., *Soc. Vert. Palaeont. Bull.*, **90**, 2 (1970).
- ¹⁷ Butzer, K. W., *Am. Anthropol.*, **73**, 1197 (1971).
- ¹⁸ Wells, L. H., *S. Afr. archaeol. Bull.*, **24**, 93 (1969).

Implications of the New Age Estimates of the Early South African Hominids

PHILIP V. TOBIAS

Department of Anatomy, University of the Witwatersrand Medical School, Hospital Street, Johannesburg

Divergences exist between recent faunal and geomorphological estimates of the age of several South African australopithecine sites. New stratigraphic evidence from Sterkfontein and Makapansgat suggests a lengthy lapse of time between cave opening and deposition of dated fossil bones, and thus provides a basis for explaining the apparent discrepancies. The young date inferred for the Taung skull makes it up to 2 m.y. more recent than *Australopithecus africanus* from the Transvaal caves. As Taung is clearly not a member of *Homo erectus*, it is reasonable to hypothesise that it may be a later survivor of *A. robustus*.

UNTIL very recently, a lack of 'absolute' or of radiometric dates for the South African early hominids has vitiated any attempt to achieve a reliable synthesis between the South and East African early hominid lineages. Faunal comparisons, often based on rather inadequate fossil assemblages, had indicated two broad groups of fauna—an earlier group, known formerly as the Sterkfontein Stage^{1,2} and subsequently as the Sterkfontein Faunal Span³, and a later group, the Swartkrans Stage, renamed the Swartkrans Faunal Span. The term faunal span was introduced to connote both a particular faunal assemblage and the temporal duration of its existence. The faunas from Sterkfontein, Makapansgat and Taung had been provisionally assigned to the earlier span and those of Swartkrans and Kromdraai to the later span^{4,5}.

Up to the middle nineteen-sixties, these intraregional faunal comparisons had led to the view that the South African sites could be arranged, from oldest to youngest, as

follows: Taung→Sterkfontein→Makapansgat→('Sterkfontein Extension Site')→Swartkrans→Kromdraai.

Wells has, however, doubted for some time^{6,7} whether the faunal evidence justifies the view that Taung with its type specimen of *Australopithecus africanus* is indeed the earliest South African australopithecine site. The hominid-associated fauna could just as readily be equated with that of Swartkrans or even of Kromdraai: and most of the identified faunal elements belong to parts of the Taung deposit which is appreciably older than the *Australopithecus* breccia. The latest check-list of cercopithecoids⁷ shows the significant absence from Taung of *Parapapio broomi*—which is the commonest cercopithecoid at Makapansgat and Sterkfontein. Moreover, there are two species of *Papio* at Taung, another two at Kromdraai and one at Swartkrans, but none at Makapansgat or Sterkfontein. It would certainly seem that the primates in the Taung cercopithecoid breccia are younger than those of the Sterkfontein Faunal Span. The analyses of Peabody⁸ and Butzer⁹, in turn, agree that the cercopithecoid breccia is appreciably older than the *Australopithecus* breccia at Taung: Butzer places them in two successive phases of one geomorphological cycle, namely Norlim 2 and 3. So the Taung hominid is younger, by a still greater margin, than the Sterkfontein Faunal Span.

Until 1970, the seriation of South African early hominid sites was based on such purely relative faunal dates. Meantime, faunal successions in East Africa were being calibrated with some precision by suites of potassium-argon dates. The next logical step was to attempt indirect dating of the South African sites by correlation of their faunas with the well-calibrated lineages of East African fauna. This was first attempted by Cooke¹⁰, who provisionally suggested that the Sterkfontein and Makapansgat breccias may be about twice as old as has hitherto been thought, possibly "in the vicinity of 2.5 to 3.0 million years old". This was based on a comparison of the fossil suids from both South African sites and of *Elephas recki* from Makapansgat (*contra*¹¹) with their well-dated counterparts from East Rudolf and Omo (Shungura Formation). Cooke's view is supported by Maglio¹², who feels that although the faunal evidence is far from adequate and further studies are needed, both Maka-

pansgat and Sterkfontein seem to correlate best with the East African faunal succession of about the middle Shungura and lowest Koobi Fora formations^{13,14}. In terms of absolute chronology, Maglio believes that 2.5 m.y. is a fair estimate of the age of these faunas. Similarly, Cooke¹⁰ has suggested, on faunal comparisons, that Swartkrans is at least as old as Bed I, Olduvai (~1.8 m.y. for the Upper Member of Bed I). These tentative faunal datings have suggested that the deposits of Makapansgat and Sterkfontein are much older than had been supposed, whereas that of Taung seems to be far younger.

The new geomorphological studies, carried out by Partridge and announced in this issue of *Nature*¹⁵, as well as those of Butzer⁹, have provided the first guarded corroboration of the faunal dates provisionally claimed by Cooke and Maglio. In spite of their use of very different approaches, both Partridge and Butzer have arrived at a relatively young age for the Taung deposit. Moreover, Partridge has provided the first evidence suggesting approximate dates for the opening of the Makapansgat (3.7 m.y.), Sterkfontein (3.3 m.y.) and Swartkrans (2.6 m.y.) caves.

These admittedly conjectural estimates suggest that the Makapansgat cave opened earliest; that of Sterkfontein about 400,000 yr later, and that of Swartkrans some 700,000 yr thereafter. It has not yet been possible to date the Kromdraai fissure deposits by these methods; Taung would be not older than Swartkrans and Kromdraai on Butzer's analysis, and as young as <0.87 m.y. BP by Partridge's approach: that is, about 1.0 m.y. younger than Swartkrans.

Partridge's method of dating is still, of course, exploratory and in its application to this problem as yet unconfirmed. Even if the method is still subject to refinement, it may be of value here to examine the degree to which his provisional dates fit other known facts and interpretations. Such a preliminary assay may, indeed, assist in putting Partridge's method to the test.

Makapansgat and Sterkfontein

Non-calibrated faunal comparisons among the South African sites had already led Wells⁵ to infer that some of the many points of difference between the faunas of Makapansgat and Sterkfontein might plausibly suggest that Makapansgat is the older of the two. This inference would now seem to gain support from Partridge's estimates for the opening of the caves, namely 3.7 and 3.3 m.y. respectively.

As has been shown, comparisons with the calibrated fauna of East Africa point to 2.5 m.y. or 2.5–3.0 m.y. for Makapansgat and Sterkfontein. There is thus a discrepancy between geomorphological and faunal estimates of 1.2–0.7 m.y. for Makapansgat and of 0.8–0.3 m.y. for Sterkfontein. It may pertinently be enquired if these are real discrepancies or technical ones. On the one hand, the divergences may be the consequence either of a methodological difficulty or lack of refinement in the geomorphological approach used, or of a problematical correlation, or of an error of faunal sampling. On the other hand, the departure between the two estimates may reflect a long lapse of time between the earliest opening of the caves and the deposition of the fossils which have been correlated with East African lineages.

Recent work at Sterkfontein¹⁶ has demonstrated the existence of an earliest bone-bearing breccia under the thick travertine once regarded as the floor of the main deposit (ref. 17, and unpublished work of T. Partridge, A. B. A. Brink and A. R. Hughes). This breccia may extend downwards for a considerable depth into the underground cave system (personal communication from J. Wilkinson). The accumulation of this lowest breccia followed the opening of the cave, but preceded the deposition of the stratigraphically much higher fauna on which the faunal dating has been based. In other words, the early stages in the

accumulation of the Sterkfontein deposit may have lasted far longer than had been imagined. This new stratigraphic evidence accords well with the lapse of a lengthy interval between the first opening of the cave (estimated at 3.3 m.y.) and the accumulation much higher in the deposit of the fossil bones on which the provisional faunal dating (2.5–3.0 m.y.) is based. Already, Butzer¹⁸ has inferred that "the Transvaal cave fills record, at the very least, several tens of thousands of years . . . and, quite possibly, several hundreds of thousands of years". In the light of this stratigraphic evidence, Partridge's estimate of a date of the order of 3.3 m.y. for the first opening of the cave is not wholly unexpected; nor is it incompatible with the provisional faunal date based on suids.

At Makapansgat, it was until recently uncertain whether there was comparable stratigraphic evidence to support a lengthy interval between the date estimated for the opening of the cave (3.7 m.y.) and the deposition of suid and elephant bones on which the tentative faunal dating (2.5–3.0 m.y.) has been based. During excavations at Makapansgat Limeworks in July 1973, I located a considerably older, lower breccia. In the Lower Cave, a conspicuous sheet of travertine has for long been regarded as lying on the original floor of the cave. Above this travertine is an accumulation of red, water-laid breccias, overtopped by the bone-rich grey breccia (which has yielded most of the hominids and datable fauna), which in turn is succeeded by the cercopithecoid-rich pink breccia, on which lies a surviving scale of the dolomite roof. The fauna used for comparison with the East African suids and proboscideans derives from layers well above the travertine floor. During our most recent excavations, in the lowest-lying parts of the cave, I located a succession of breccias and travertines below the supposed basal travertine; some of these oldest breccias proved to be bone-bearing.

Thus, at Makapansgat, as at Sterkfontein, there is stratigraphic evidence suggesting a lengthy lapse of time between the earliest opening of the cave and the deposition of those bones used for comparison with East African faunas. This evidence would suggest a much older date for cave opening than for the calibrated fauna. Against this background, Partridge's suggestion that the date of cave opening was of the order of 3.7 m.y. is not wholly unlikely; nor does it seriously contradict the faunal date of 2.5–3.0 m.y. tentatively inferred by Cooke and Maglio.

Both at Makapansgat and at Sterkfontein, it now seems not altogether improbable that the filling of the caves may have occupied between 0.3 and 1.2 m.y. The upper part of this range of time is sufficiently long to make it likely that significant changes of fauna and perhaps of culture may be entombed within the deposit. Already there is some evidence to suggest a succession, at both Sterkfontein and Makapansgat.

The earlier age inferred for the Makapansgat deposit seems to be in keeping with the evidence of the hominids themselves. The Makapansgat hominids are characterised by 'gracile' or *A. africanus* features, but include as well some *A. robustus* traits in crania, jaws and teeth, to which attention was drawn in 1967: "In these respects, the Makapansgat specimens seem to show a somewhat nearer approach to *A. robustus* than do the Sterkfontein specimens"¹⁹. This led me subsequently to suggest tentatively that more than one species of hominid is present at Makapansgat^{20,21}. From a study of the mandibles, Aguirre²² has since claimed that the Makapansgat juvenile jaw, MLD 2, indubitably represents a young male *A. robustus*.

In the light of the new tentative age determinations for Makapansgat, it might be suggested that a *mélange* of *A. africanus* and *A. robustus* features might have been expected in an early population of *A. africanus*, close to the point of speciation into robust and gracile species. As an early population, it is reasonable, in Darwinian and in genetic

terms, to suppose that its spectrum of anatomical polymorphisms adumbrates later speciation of the *A. robustus*-*A. boisei* lineage from a basic *A. africanus* lineage.

Swartkrans

The Swartkrans Faunal Span is younger than the Sterkfontein Faunal Span. The Swartkrans fauna itself is considered older than that of Kromdraai and than the Cornelia Faunal Span (from Cornelia, Orange Free State)⁵. Partridge's new study has tentatively inferred a date of 2.6 m.y. for the opening of the Swartkrans cave, and Cooke, on the fauna, has estimated an age of roughly 1.8 m.y. at least. Again there is an apparent discrepancy, this time of about 0.8 m.y., between the two estimates: but both confirm what intraregional faunal comparisons have indicated, that Swartkrans is younger than Makapansgat and Sterkfontein. Brain's excavations at Swartkrans^{23,24} may yet reveal whether there is a stratigraphic explanation for the gap between the two claimed dates at this site.

Taung

The type-site of *Australopithecus africanus*, Taung, was long regarded as the oldest of the South African early hominid sites. Wells pointed out that the species most closely associated with the juvenile hominid skull do not warrant this view, and that Taung could equate with Swartkrans or even Kromdraai^{5,6}.

Butzer and Partridge agree that Taung is much younger than Makapansgat and Sterkfontein, claiming that it is either coeval with or younger than Swartkrans-Kromdraai (Butzer), or younger than 0.9 m.y. BP (Partridge). The second estimate refers to the *terminus post quem* for the formation of the original Taung cavern: on this conjecture, it would follow that the lower, cercopithecoid breccia is somewhat younger than <0.9 m.y., and the upper, *Australopithecus* breccia would be still younger, perhaps 0.8 or even 0.7 m.y. BP. If, as Cooke suggests¹⁰, the age of the Swartkrans fauna is at least 1.8 m.y., Butzer's maximum age for Taung would be 1 m.y. earlier than Partridge's tentative estimate. On the other hand, Butzer agrees that Taung may be younger than Swartkrans—and this would reduce the discrepancy between his and Partridge's estimates. It is not possible at present to determine if any residual discrepancy would have a real, stratigraphic basis, or be only apparent, the result of errors in either or both estimates.

In any event, one can only conclude that, far from being the oldest australopithecine site in southern Africa, Taung is probably the youngest. What is more, it may compete with Chesowanja and Peninj (both just over 1.0 m.y. BP) as being the site of the most recent survival of an australopithecine anywhere in Africa. In Fig. 1, Taung has been plotted at about 0.8 m.y., with an interrupted line extending back to the approximate date of Kromdraai.

On the basis of Partridge's estimate, the Taung skull, the type specimen of *A. africanus*, represented a hominid that lived up to 2 m.y. later than the Makapansgat and Sterkfontein hominids, which are generally regarded as belonging to *A. africanus*. Between the two periods of 2.5–3.0 m.y. and 0.8 m.y., no trace of *A. africanus* has been identified in southern Africa, even though two sites (Swartkrans and Kromdraai) are probably dated to the intervening period. In East Africa, hominids datable to the intervening period have been attributed, in the main, to the robust australopithecines (the superspecies, *A. robustus*/*A. boisei*²⁵) and to *Homo* sp. Furthermore, some workers have claimed to recognise the presence of *A. cf. africanus* at Omo (chiefly isolated teeth) and at Olduvai (for example, Olduvai hominid 7, recognized by Leakey *et al.*²⁶ as the type specimen of the bigger-brained *H. habilis* and regarded by others as *A. habilis*). Thus, the presence of *A. africanus* in East Africa between 2.5–3.0 m.y. and 0.8 m.y. remains disputed and

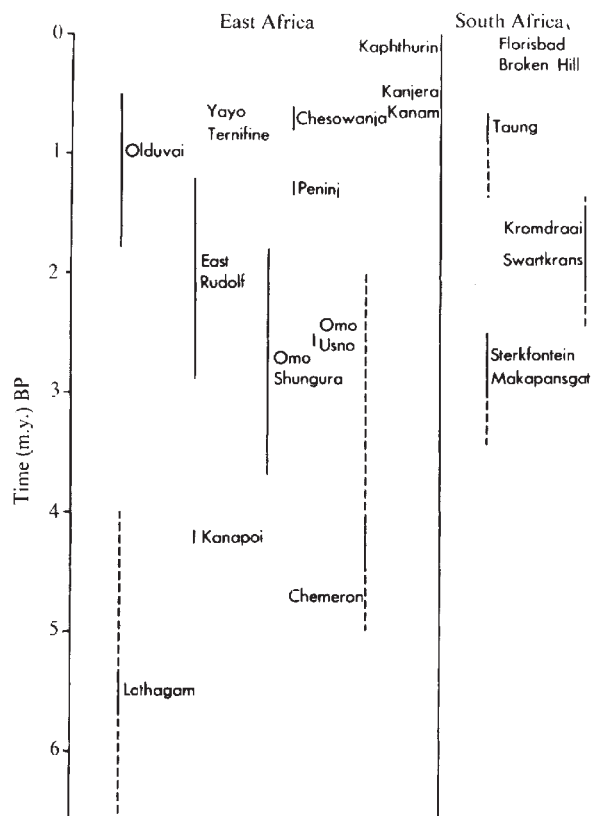


Fig. 1 Provisional temporal distribution of East African hominids (after Maglio¹²) and of South African hominids (based on the new dates).

equivocal. The most convincing East African claimant for membership of *A. africanus* is the far older Lothagam mandible²⁷ dated to about 5.0–5.5 m.y.²³.

Phylogenetic Implications

Figure 1 depicts the dates of the South African sites, based on the latest provisional faunal datings and modified somewhat by the geomorphological estimates of Partridge. These are compared with those for East Africa as formulated by Maglio¹².

In Fig. 2, an attempt has been made to represent the various hominid taxa living at 0.5 m.y. intervals in South and East Africa. Although there are several different classifications of hominids, I have used a bigeneric one, because the recognition of two Plio-Pleistocene hominid genera, *Australopithecus* and *Homo*, enjoys the greatest consensus at the present time^{25,29}. Furthermore, I have used a division of *Australopithecus* into *A. africanus*, *A. robustus* and *A. boisei*, the last two being considered as two allopatric species forming one superspecies²⁵; and of *Homo* into *H. habilis*, *H. erectus* and *H. sapiens*.

In Fig. 3 I have made a new reconstruction of the phylogeny of the Plio-Pleistocene hominids, using the new tentative South African hominid age estimates. The early origin of *Homo* in the figure is based on the probable date of 2.9 m.y. for R. E. F. Leakey's new cranium, KNM-ER 1470, found below the KBS tuff of East Rudolf³⁰. From my examination of this important find, kindly permitted by Mr Leakey, I am impressed by the morphological resemblances between ER 1470 and the crania assigned to *H. habilis*. Tentatively, I accept Leakey's attribution of ER 1470 to *Homo* sp.: as a provisional judgment, it may even extend our concept of the range, in time and morphology, of *H. habilis*.

If the late date proposed for the Taung child is more or less correct, its only known contemporaries are *Homo* (most

probably *H. erectus*) and the last-surviving members of the robust australopithecine superspecies (Fig. 2). The inferred date of about 0.8 m.y. BP makes it >1.0 m.y., and probably about 2.0 m.y., more recent than the latest of the other African fossils assigned to *A. africanus*.

Hence, it may well be questioned whether the Taung child does in fact represent *A. africanus*, in the sense in which this taxon is defined from Makapansgat and Sterkfontein. The possibility of isolated relict populations of *A. africanus* having survived in the southerly cul-de-sac of Africa for 1 to 2 m.y. after the species had disappeared elsewhere, or had given rise to the earliest species of *Homo* in Africa, cannot of course be excluded. On the other hand, because its small brain³¹ and its dental characters would exclude it from *H. erectus*, it must seriously be considered whether the Taung child is not a late surviving member of *A. robustus* or *A. cf. robustus*. Although nearly 50 yr have elapsed since its discovery³², it is true to say that the Taung skull has never yet been fully analysed and described. In my laboratory, G. R. Sperber has recently made X rays of the Taung maxilla and mandible which reveal the unerupted permanent premolars and second molars. A careful radiological study of these tooth germs, and the possible extraction of two of them, may provide additional and perhaps diagnostic dental evidence on the affinities of the Taung child. Certainly, the recent discovery of new juvenile specimens of *A. robustus*²⁴ and other early hominids would make a definitive study of the Taung skull both timely and potentially most rewarding. It is hoped that such a study would provide evidence to confirm—or refute—the hypothesis that the Taung child represents a member of *A. robustus*.

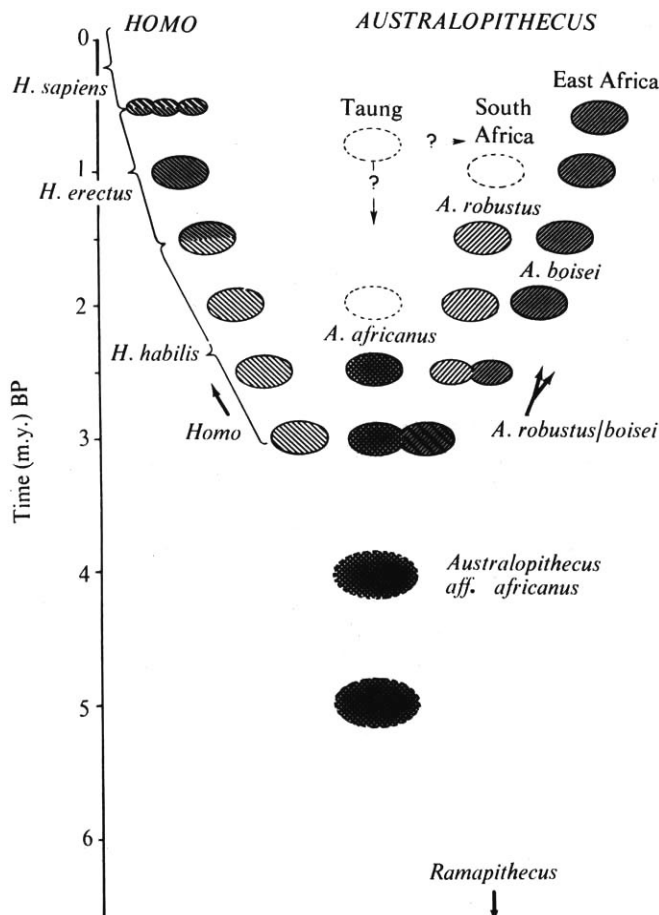


Fig. 2 Hominid populations in Africa at various times in the Plio-Pleistocene. The oval figures at each time level represent the various systematically identified, synchronic hominid taxa living in Africa. The horizontal distance between a pair of ovals at the same time level is roughly proportional to the morphological and taxonomic distance between the populations or taxa represented by the ovals.

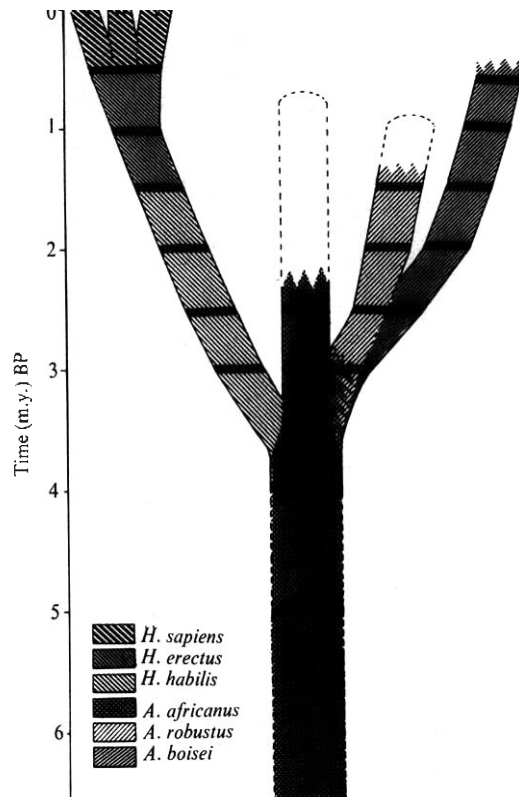


Fig. 3 Provisional phylogenetic tree of the Plio-Pleistocene Hominidae. The possible extension of *A. africanus* and/or *A. robustus* to a very recent period (<1.0 m.y. BP) is suggested as two alternative means of accommodating the Taung skull at its new young date (0.7–0.8 m.y. BP).

Taxonomic Implications

If the Taung skull has to be removed from the paradigm of *A. africanus* and included in that of *A. robustus*, the specimen's place as the holotype of *A. africanus* would have to be revised. In any event, since the nominal species, *A. africanus*, was based on an immature stage lacking, to a certain extent, good diagnostic characters, the designation of a lectotype under Article 74 of the International Code of Zoological Nomenclature would seem desirable. The inclusion of the Taung skull in *A. robustus* would not automatically lead to its designation as the type specimen of this species: its juvenile status would probably preclude it from being so designated and the holotype of *A. robustus* would remain the skull, teeth and postcranial bones of the adult specimen, TM 1517, from Kromdraai, Transvaal.

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- Wells, L. H., *Ann. Cape Prov. Mus.*, **11**, 37 (1962).
- Ewer, R. F., and Cooke, H. B. S., in *Ecological Studies in Southern Africa* (edit. by D. H. S. Davis), 415 (W. Junk, The Hague, 1964).
- Cooke, H. B. S., in *Background to Evolution in Africa* (edit. by Bishop, W. W., and Clark, J. D.), 935 (University of Chicago Press, Chicago and London, 1967).
- Cooke, H. B. S., *Q. Rev. Biol.*, **43**, 234 (1968).
- Wells, L. H., *S. Afr. archaeol. Bull.*, **24**, 93 (1969).
- Wells, L. H., in *Background to Evolution in Africa* (edit. by Bishop, W. W., and Clark, J. D.), 935 (University of Chicago Press, Chicago and London, 1967).

- ⁷ Freedman, L., *Palaeont. afr.*, **13**, 109 (1970).
- ⁸ Peabody, F. E., *Bull. geol. Soc. Am.*, **65**, 671 (1954).
- ⁹ Butzer, K., *Curr. Anthropol.* (in the press).
- ¹⁰ Cooke, H. B. S., *News Bull. Soc. vertebr. Paleont.*, **90**, 2 (1970).
- ¹¹ Wright, G. A., and Skaryd, S., *Nature*, **237**, 291 (1972).
- ¹² Maglio, V. J., *Trans. Am. Phil. Soc. (N.S.)*, **63** (3), 1 (1973).
- ¹³ Maglio, V. J., *Nature*, **231**, 248 (1971).
- ¹⁴ Maglio, V. J., *Nature*, **239**, 379 (1972).
- ¹⁵ Partridge, T. C., *Nature*, **246**, 75 (1973).
- ¹⁶ Tobias, P. V., and Hughes, A. R., *S. Afr. archaeol. Bull.*, **24**, 158 (1969).
- ¹⁷ Tobias, P. V., *A. Rev. Anthropol.* (in the press).
- ¹⁸ Butzer, K. W., *Am. Anthropol.*, **73**, 1197 (1971).
- ¹⁹ Tobias, P. V., *Olduvai Gorge, 2: The Cranium and Maxillary Dentition of Australopithecus (Zinjanthropus) boisei*, 264 (Cambridge University Press, Cambridge, 1967).
- ²⁰ Tobias, P. V., *New African Evidence on Human Evolution* (Wenner-Gren Foundation Supper Conference, New York City, April 1968).
- ²¹ Tobias, P. V., *Yearb. phys. Anthropol.*, 1967 (edit. by S. Genoves), 24 (1969).
- ²² Aguirre, E., *Identificacion de "Paranthropus" en Makapansgat (Cronica del XI Congreso Nacional de Arqueologia, Merida 1969)*, 98 (1970).
- ²³ Brain, C. K., *S. Afr. J. Sci.*, **63**, 368 (1967).
- ²⁴ Brain, C. K., *Nature*, **225**, 1112 (1970).
- ²⁵ Tobias, P. V., in *L'Origine dell' Uomo* (Accademia Nazionale dei Lincei, Rome, in the press).
- ²⁶ Leakey, L. S. B., Tobias, P. V., and Napier, J. R., *Nature*, **202**, 7 (1964).
- ²⁷ Howell, F. C., in *Calibration of Hominoid Evolution* (edit. by Bishop, W. W., and Miller, J. A.), 487 (Scottish Academic Press, Edinburgh, 1972).
- ²⁸ Cooke, H. B. S., and Maglio, V. J., in *Calibration of Hominoid Evolution* (edit. by Bishop, W. W., and Miller, J. A.), 487 (Scottish Academic Press, Edinburgh, 1972).
- ²⁹ Tobias, P. V., in *Taxonomy and Phylogeny of Old World Primates with Reference to the Origin of Man* (edit. by Chiarelli, B.) (Rosenberg and Sellier, Turin, 1968).
- ³⁰ Leakey, R. E. F., *Nature*, **242**, 447 (1973).
- ³¹ Holloway, R. L., *Science, N.Y.*, **168**, 966 (1970).
- ³² Dart, R. A., *Nature*, **115**, 195 (1925).

LETTERS TO NATURE

PHYSICAL SCIENCES

Ionospheric Effects of Cosmic γ -ray Bursts

THE recent discovery of bursts of γ rays of cosmic origin¹ has led to the suggestion that these bursts might produce observable ionospheric effects. Since γ rays are absorbed at a relatively low altitude when compared with most other types of ionising radiation, the thought was that these bursts would produce additional ionisation which might be strong enough to affect v.l.f. radio propagation data. If so and if data from several paths were obtained, it might be possible to obtain information about the location of the sources. Directional information from the Vela satellites, when obtained at all, contains an inherent ambiguity.

The calculations reported here were carried out by means of a Monte Carlo code which was developed to calculate X-ray and γ -ray transport in a model atmosphere. The model used is based on the 1962 COESA standard atmosphere². In the code it is described by 120 plane parallel layers, with each layer 1 km thick. Within each layer the density is assumed to decrease exponentially with altitude, and is described by a tabulated initial density and scale height for each zone. Above 120 km the atmosphere is assumed to provide negligible photon attenuation. The atmosphere is considered to have constant chemical composition, with photon cross sections as given by Storm and Israel³. Up to 120 km this approximation is quite good, but begins to become invalid at higher altitudes. Electron transport is neglected; that is, a photon which is absorbed or loses energy in a Compton collision is assumed to deposit all this energy in the 1 km zone in which the interaction occurs. This is a valid approximation, since at altitudes of from 20 to 40 km, the region in which most of the interactions take place, the thickness of the individual layers ranges from 9 g cm⁻² to 0.4 g cm⁻². The range⁴ of the highest energy Compton electron which can be produced is approximately 0.35 g cm⁻²; the vast majority of the electrons have considerably lower energy.

A calculation of energy deposition requires a knowledge of the incident spectrum. Spectral information from the Vela satellites is only qualitative, being based on total counts in two overlapping channels, with energy responses covering the

ranges from 0.2 to 1 MeV and 0.3 to 1.5 MeV, respectively. But since the original announcement of the Vela discovery, it has been learned that several of these events were also observed by the Goddard Space Flight Center γ -ray detector on board IMP-6 (ref. 5), and that one was seen by the two USCD X-ray telescopes on board OSO-7 (ref. 6). The IMP-6 and OSO-7 instruments provide overlapping spectral information for one event, 72-4 in the Vela catalogue, covering the energy range from 11 to 800 keV. This spectrum was used in the calculations reported here.

Several simplifying assumptions about the nature of the source were made in carrying out the calculations. The spectrum was assumed to be independent of time, although for most of the events observed by the IMP-6 detector the spectrum seems to soften somewhat with time. The time history as observed by both the Vela and OSO-7 satellites shows complex structure, but in the calculations it was assumed that the burst rose above background instantaneously and decayed exponentially, with a time constant of 20 s.

Table 1 Atmospheric Energy Deposition by Cosmic γ -ray Burst 72-4, having Total Incident Energy per Unit Area of 3×10^{-4} erg cm⁻²

Zenith angle (degrees)	Peak energy deposition (eV cm ⁻³)	Altitude of peak energy deposition (km)
0	85	28
6	86	29
12	85	29
18	82	29
24	76	29
30	72	29
36	66	30
48	49	31
60	33	32
72	16	33
84	3	40

Peak total energy deposition for various zenith angles is shown in Table 1. The ionisation is not very strongly dependent on zenith angle over quite a wide range. This particular event is not the strongest that has been observed in terms of total energy incident on the atmosphere, but it is not untypical. The peak energy deposition of approximately 85 eV cm⁻³ corresponds roughly to an electron production of 2 or 3

electrons cm^{-3} . It occurs at an altitude considerably lower than what is usually considered the lowest part of the daytime D layer. At that altitude the only competing process is ionisation by galactic cosmic rays⁷. In fact, at high geomagnetic latitudes the magnitudes of the two effects are comparable. This is shown in Fig. 1, where the steady state electron density produced by galactic cosmic rays at geomagnetic latitude greater than 70° is compared with the total number of electrons produced over a period of 80 s by γ -ray burst 72-4.

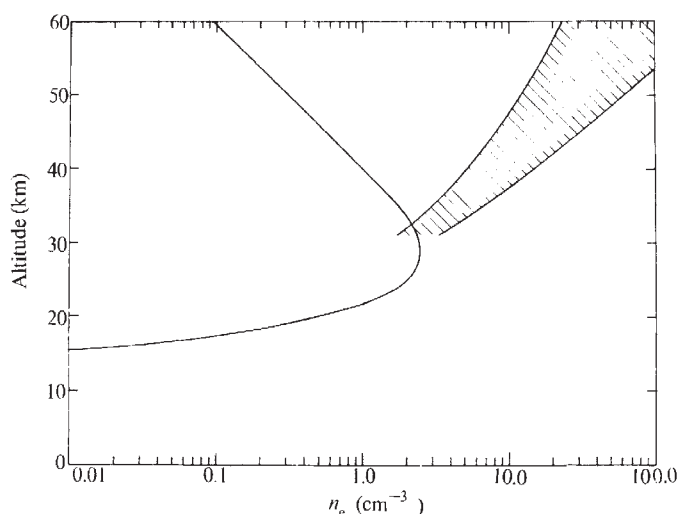


Fig. 1 Total electron density produced by cosmic γ -ray burst 72-4 compared with the steady state electron density produced by galactic cosmic rays. Cross-hatched area covers extremes of night-time solar maximum and day-time solar minimum as calculated by Webber⁷ for geomagnetic latitude greater than 70° .

So the best chance of observing these bursts would be at low geomagnetic latitudes, where the ionisation induced by galactic cosmic rays would be of a lesser magnitude than that produced by the burst. The burst would produce only a very transient effect, since at the altitudes at which the peak ionisation occurs the effective recombination coefficient is a very rapidly increasing function of decreasing altitude⁷. This requires that data be recorded on a finer time scale than is usually the case when, for example, the effects of geomagnetic activity are being studied⁸.

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ROBERT T. BROWN

University of California,
Los Alamos Scientific Laboratory,
Los Alamos, New Mexico 87544

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¹ Klebesadel, R. W., Strong, I. B., and Olson, R. A., *Astrophys. J. Lett.*, **182**, L85 (1973).

² Committee on Extension to the Standard Atmosphere, *US Standard Atmosphere* (US Government Printing Office, Washington, DC, 1962).

³ Storm, E., and Israel, H. I., *Los Alamos Scientific Laboratory Report*, LA-3753 (1967).

⁴ Pages, L., Bertel, E., Joffe, H., and Sklavenitis, L., *Atomic Data*, **4**, 1 (1972).

⁵ Cline, T. L., Desai, U. D., Strong, I. B., and Klebesadel, R. W., *Astrophys. J. Lett.*, **185**, L1 (1973).

⁶ Wheaton, W. A., Ulmer, M. P., Baity, W. A., Datlowe, E. W., Elcan, M. E., Peterson, L. E., Strong, I. B., Klebesadel, R. W., Cline, T. L., and Desai, U. D., *Astrophys. J. Lett.*, **185**, [L77] (1973).

⁷ Webber, W., *J. geophys. Res.*, **67**, 5091 (1962).

⁸ Potemra, T. A., and Rosenberg, T. J., *J. geophys. Res.*, **78**, 1572 (1973).

Structure of the Terrestrial Planets

BULLEN¹ has suggested that to reconcile known data for Venus, Earth and Mars with the hypothesis that they have the same overall composition, the outer core of the Earth may be composed of Fe_2O . The principal reason seems to be the resolution of an alleged discrepancy between the modern solid radius of Venus determined by radar², $6,053.7 \pm 2.2$ km and the value of 6,270 km computed by Bullen on the phase-change hypothesis for the core³. But this latter value was arrived at on the basis of somewhat approximate calculations, whereas more accurate calculations⁴ find no discrepancy of any significance in interpreting Venus as possessed of three main zones with mechanical-elastic properties the same as in the corresponding zones of the Earth. In such calculations some allowance for the existence in Venus of a small inner core could readily be made, but it is clear that no adjustment for such an inner core, which in the Earth has volume only 0.76% of the whole, could seriously affect the computed value of the overall radius. For if the inner core were completely annihilated and the Earth contracted down geometrically without change of volume of the remainder, the outer radius would shrink only by 16 km, so that averaging of its properties with those of the main bulk of the core would produce negligible change of radius, whereas Bullen's discrepancy is over 200 km.

Although the possibility that the core consists of Fe_2O cannot be ruled out, there seems to be no need to postulate this, or any specific composition as to its chemical nature, when it comes to the question of calculation of the internal structures of other planets on the hypothesis that they have the same overall composition as the Earth itself; it is only the elastic constants and uncompressed density of the material that enter such calculations. Even if the properties of Fe_2O at pressures in excess of 10^{12} dyne cm^{-2} and temperatures of say 5,000 K ($\pm 2,000$ K) could be accurately calculated, its identification as core material could be based only on the agreement of its elastic constants and density with the known values derived for core material from the seismic data. Because these latter values are reliably known anyway for the Earth, they can be transferred immediately for use in regard to the other planets, without raising the question of absolute composition. When that is done the modern radar value for the solid radius of Venus leads to no difficulty in arriving at an acceptable internal structure. On the other hand, there could be no guarantee that other compounds may not have the same elastic properties and density as Fe_2O at the conditions concerned, and thus making detailed identification superfluous.

Adopting the round figure of 6,055 km for the radius, the model that is most plausible on the phase-change hypothesis has the following properties⁴: m_c =mass of core= 1.20×10^{27} g=0.247 mass of Venus; ρ_c =central density= 11.45 g cm^{-3} ; p_c =central pressure= 2.70×10^{12} dyne cm^{-2} ; r_c =radius of core=3,032 km=0.5008 radius of Venus; p_{12} =pressure at core/mantle boundary= 1.23×10^{12} dyne cm^{-2} ; r_m =radius to mantle/outer-shell boundary=5,540 km=0.915 R_V ; m_{12} =combined mass of core and mantle= 4.11×10^{27} g=0.844 M_V ; p_{23} =pressure at top of mantle= 0.16×10^{12} dyne cm^{-2} ; ρ_m =mean-density of whole planet to solid surface= 5.233 g cm^{-3} ; f =moment-of-inertia factor=0.3424; W =total (negative) gravitational energy= 0.1703×10^{40} erg.

If the pressure p_{12} at the core/mantle interface and the pressure p_{23} at the mantle/outer-shell interface were both assigned, then a unique surface radius r_s would result. Conversely, if the radius is regarded as known, then only one or other of these pressures is disposable. Where p_{23} is concerned, there is experimental evidence⁵ that the phase change involved is influenced by the temperature in the sense that higher pressure is needed to bring it about at higher temperature. In the Earth, the value of p_{23} is at present about 0.14×10^{12} dyne cm^{-2} . Calculations for Venus have been made³ covering a range of values of p_{23} from 0.12 to 0.16 ($\times 10^{12}$ dyne cm^{-2}),

and these show that the pressure p_{12} at the boundary of the core ranges only from about 1.22 to 1.27 ($\times 10^{12}$ dyne cm^{-2}), whereas the pressure at the core boundary in the Earth is 1.37 ($\times 10^{12}$ dyne cm^{-2}). If this boundary is regarded as an abrupt change of composition, then no special inference could be drawn from these lower pressures for Venus, but if it represents a phase change to metallic-liquid form, then this change being such that its production at lower pressure requires higher temperature, the inference would be that Venus is at somewhat higher internal temperature than is the Earth at this boundary. This is consistent with heating through radioactivity requiring longer for the central temperature to rise to an adequate level before bringing on the sudden Ramsey collapse^{6,7}, with part of the vast gravitational energy thereby released further increasing the temperature. For these reasons, a higher temperature might well also be implied for the mantle/outershell boundary, and the value 0.15 ($\times 10^{12}$ dyne cm^{-2}) was therefore selected to accord with this in settling the above most likely model. Venus would reach this stage later than the Earth, and its whole internal development may be lagging behind that of the Earth by some fraction of an aeon. Higher internal temperature for Venus might also receive some contribution through its greater proximity to the Sun coupled with a long-sustained greenhouse effect.

The proportion by mass of Venus in its core is seen to be 24.7%, compared with 31.4% for the Earth, and with the radius fixed at 6,055 km no serious change of this percentage is possible for any reasonable adjustment of the interface pressures. (If the radius is taken to be larger, the percentage is reduced.) Indeed, if Venus were to have the terrestrial properties by mass in its core, the radius could not be greater than about 5,980 km—more than 70 km less than the observed value. On the hypothesis that the terrestrial planets and the Moon have similar compositions, homogeneous on a sufficiently large scale, so that the known elastic constants and uncompressed densities for the various zones of the Earth can be utilized to compute models of these bodies, then the following scheme of possible explanation of their structures, as regards the presence or otherwise of a core, emerges from the presently known values of their masses and radii (Table 1).

Table 1 Properties of Terrestrial Planets

	Mass	Mean density	Percentage mass in core	Possible explanation
Earth	1.0	5.52	31.4	Iron or phase change
Venus	0.815	5.23	24.7	Iron or phase change
Mars	0.108	3.92	0.0	No metallic core
Mercury	0.055?	5.42?	60.0	Iron core only
Moon	0.012	3.35	0.0	No metallic core

For the purpose of computing the percentages of Table 1, no discrimination need be made between the time-honoured iron hypothesis and the Ramsey phase-change hypothesis for the terrestrial core, because the parameters needed depend solely on the seismic data for the Earth. On the first hypothesis, comparing the core masses with that of the Earth, each of the other bodies is seen from Table 1 to present a problem in varying degree. It would be necessary to suppose that Venus had distinctly smaller proportion of iron than the Earth, whereas the slightly more distant Mars and the closely adjacent Moon, without iron cores at all, would have considerably less proportion of iron overall and the present mass and radius of Mercury would require this planet to be a most remarkable object with about 60% of its mass in the form of an iron core and its whole iron content presumably greater still. On the phase-change hypothesis, however, these striking differences between the bodies follow immediately as a direct consequence of the internal pressures resulting from the differences in mass, the only difficulty being the egregious Mercury.

The value for the mean density of Mercury depends not only on that found for the mass but, even more sensitively, on that for the radius. Modern determinations, which are made by a combination of radar measures and dynamical theory, may nevertheless be influenced from the outset through the adoption, as starting values for linearized correction, of the mass originally announced by Rabe⁸ in 1950 from the orbit of Eros, and of the optically observed radius. Just as, to take a simple example, Newton's method may fail to find the appropriate root of an equation if the starting value selected happens to lie too close to another inapplicable root, so in the present case the situation may be such that arrival at the correct mass may automatically be prevented. This 1950 value for the mass has recently been practically withdrawn as to its reliability by Rabe⁹, who on further investigation of the method finds the (reciprocal) mass in fact to be subject to a probable error in excess of 50%. The difficulty of determining the radius of a small object lying so close to the Sun is of course so generally appreciated as to call for no further comment here.

The importance of the forthcoming Venus-Mercury flyby for resolving the seemingly anomalous structure of Mercury is self evident. Until confirmation by such means is available it seems scarcely worthwhile endeavouring to explain how it may have come about, or further still how the apparently strange composition may affect theories of the origin of the planets. On the basis of the phase-change hypothesis, a mean density for Mercury less than that of Mars would seem a reasonable expectation.

R. A. LYTTLETON

*Institute of Astronomy,
Cambridge CB3 0HA*

Received May 29, 1973.

- ¹ Bullen, K. E., *Nature*, **243**, 68 (1973).
- ² Ash, M. E., *et al.*, *Science*, **160**, 985 (1968).
- ³ Bullen, K. E., *Mon. Not. R. astr. Soc.*, **110**, 256 (1950).
- ⁴ Lyttleton, R. A., *Astrophys. Space Sci.*, **5**, 18 (1969).
- ⁵ Ringwood, A. E., *Geochim. cosmochim. Acta*, **26**, 457 (1962).
- ⁶ Ramsey, W. H., *Mon. Not. R. astr. Soc.*, **110**, 325 (1950).
- ⁷ Lyttleton, R. A., *Proc. R. Soc., A*, **296**, 285 (1965).
- ⁸ Rabe, E., *Astr. J.*, **55**, 112 (1950).
- ⁹ Rabe, E., and Francis, M. P., *Astr. J.*, **72**, 316 and 862 (1967).

PROFESSOR BULLEN replies: The statement¹ that "The principal reason for [the Fe₂O proposal] seems to be the resolution of an alleged discrepancy between the modern solid radius [R_V , say] of Venus determined by radar . . . and the value computed by Bullen on the phase-change hypothesis" does not put the facts in correct perspective.

I gave² four independent objections to the phase-transition theory, the discrepancy over R_V being only one item. More potent objections³ are: (a) the difficulty of reconciling the phase-transition theory with the large density jump at the Earth's mantle core boundary; (b) the failure to obtain support for that theory from shockwave high-pressure experiments. These objections have caused perhaps a majority of geophysicists to "rule out" the theory. My estimate is that the above objections make it desirable to explore alternatives, of which the Fe₂O theory is one. That theory, in contrast to the phase-transition theory, has the special strength that it avoids the potent objections ((a) and (b)) while yielding fair agreement with the relevant observational data. Lyttleton¹ disregards this vital point.

I do not agree that my 1950 estimate³ of 6,270 km for R_V , which assumed the phase-transition theory, is less dependable than Lyttleton's later calculations^{4,5}. I regard the opposite as the case. My calculations used numerical integration along with direct numerical detail on the Earth's density and pressure

distributions, and the procedures were formally reliable within ~ 10 km for R_V . In contrast, Lyttleton's calculations introduced a number of implicit assumptions which I did not find necessary and which distorted the starting Earth model, making his numerical results distinctly less reliable than mine (see also ref. 6).

Further, Lyttleton shows a lack of grasp of what the Fe_2O theory does in respect of planetary core radii. He refers to the volume of the Earth's inner core being only 0.76% of the volume of the Earth and infers that the presence or absence of an inner core in Venus would therefore have negligible effect on the estimated R_V . What is not appreciated is that the very large reduction of order 200 km from my estimate of R_V on the phase-transition theory to that on my Fe_2O theory arose principally because the Fe_2O theory entails relatively larger inner cores in smaller planets; for Venus, the inner core is given as not merely relatively larger than in the Earth, but actually as having nearly six times the volume of the Earth's inner core. On the Fe_2O theory, the Venus inner core has 4.4% of the volume of Venus. A consequence is that the change in the estimated R_V on the two theories is by no means negligible.

Why then does the Venus model detailed in ref. 1 have a value so close to the present observed value of $\sim 6,050$ km. I attribute this partly to some of the unreliable features of Lyttleton's procedures (see ref. 6), partly to *ad hoc* assumptions made by Lyttleton, and partly to his provision for possible temperature differences; this provision places an additional parameter at his disposal in a way that amounts to a further *ad hoc* device.

A very important corollary to the substantially larger inner core for Venus entailed on the Fe_2O theory is that the outer core of Venus is small compared with the Earth's outer core. Thus (assuming the dynamo theory of planetary magnetic fields), the Fe_2O theory fits better than all other theories the failure to detect a magnetic field around Venus.

While conceding that my Fe_2O theory cannot be "ruled out", Lyttleton questions the need for postulating any specific composition in planetary interiors. I have already indicated that one important reason for proposing the Fe_2O theory is that it avoids the objections (a) and (b). Moreover, a key property of Fe_2O for present purposes is essentially physical—the instability assumed at pressures less than a critical pressure (which corresponds to that at the Earth's mantle-core boundary). It is conceivable that the Earth's outer core could be composed of another material which exhibits this instability; if such a material should be found there could be a case for its investigation in respect of planetary structure.

More serious, however, is the statement¹ that the "identification [of Fe_2O] as core-material could be based only on the agreement of its . . . density with the known values derived . . . from seismic data". No reference is made to Sorokin's calculations that Fe_2O has the assumed instability and that its density-pressure relation for conditions prevailing in the Earth's outer core matches that in recent Earth models (see, for example, ref. 8).

Lyttleton's comment on the considerable uncertainty of the observational mean density of Mercury is interesting. Because of the uncertainties it has seemed to me that all that can, on present evidence, be reasonably said on the internal structure of Mercury was said by 1952. It has long been established that "on the basis of the phase change hypothesis a mean density for Mercury less than that of Mars would appear to be a reasonable expectation" (see, for example, ref. 9).

No useful favourable test of Lyttleton's theory has emerged since it was promulgated some ten years ago, but there are² possible tests of the Fe_2O theory in the foreseeable future. I seem to differ from Lyttleton in that I do not wish to press any particular theory beyond what I regard as the balance of evidence. Should future tests favour the phase-transition theory, no one would be more delighted than myself because of my earlier association with it. But it seems to me that,

on a proper weighting of current evidence, including points (a) and (b) above, the Fe_2O theory has fewer difficulties to meet than the phase-transition theory.

K. E. BULLEN

University of Sydney,
Australia

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- ¹ Lyttleton, R. A., *Nature*, **246**, 84 (1973).
- ² Bullen, K. E., *Nature*, **243**, 68 (1973).
- ³ Bullen, K. E., *Mon. Not. R. astr. Soc.*, **110**, 256 (1950).
- ⁴ Lyttleton, R. A., *Mon. Not. R. astr. Soc.*, **129**, 21 (1965).
- ⁵ Lyttleton, R. A., *Astrophys. Space Sci.*, **5**, 18 (1969).
- ⁶ Bullen, K. E., *Mon. Not. R. astr. Soc.*, **133**, 229 (1966).
- ⁷ Bullen, K. E., *Mon. Not. R. astr. Soc.*, **109**, 689 (1949).
- ⁸ Bullen, K. E., and Haddon, R. A. W., *Phys. Earth planet. Int.*, **1**, 1 (1967).
- ⁹ Bullen, K. E., and Low, A. H., *Mon. Not. R. astr. Soc.*, **112**, 637 (1952).

Refining the Earth's Pear Shape

QUITE soon after the launching of the first artificial satellites, analysis of their orbits led to the reshaping of the world. The orbital plane of Sputnik 2 rotated less rapidly than expected, indicating¹ that the polar flattening was about 0.35% less (the polar diameter about 150 m greater) than had been thought. The variations in the perigee height of Vanguard 1, as perigee moved successfully north and south of the equator, showed² that the polar section of the geoid—the profile of the sealevel surface revealed by a cut through the poles—was not symmetrical about the equator but had a slight pear-shape tendency, with the stem at the North Pole. Floating at sealevel at the North Pole, one would be about 40 m further from the equator than an equally undaunted explorer who bored down to sealevel at the South Pole.

Since 1959 the odd zonal harmonics in the geopotential, which determine the extent of the pear shape, have been evaluated with improving accuracy and reliability, from analysis of satellite orbits at a wide variety of inclinations to the equator. But until recently no suitable satellites have been available with near-equatorial orbits or with orbits close to the critical inclination of 63.4° , where the perturbations caused by the odd harmonics become extremely large, 50 km or more. In this letter we report the results of a new determination of the odd zonal harmonics³ from the orbits of twenty-seven satellites, including two orbits close to the critical inclination, determined with the help of observations from the Hewitt cameras at Malvern and Edinburgh.

If variations with longitude are ignored, the geopotential U at an exterior point distant r from the Earth's centre and having geocentric latitude ϕ , may be expressed in the standard form¹

$$U = \mu/r \left\{ 1 - \sum_{n=2}^{\infty} J_n(R_e) P_n(\sin \phi) \right\} \quad (1)$$

where μ is the gravitational constant for the Earth ($398,601 \text{ km}^3 \text{ s}^{-2}$), R is the equatorial radius ($6,378.14 \text{ km}$) and $P_n(\sin \phi)$ is the Legendre polynomial of degree n and argument $\sin \phi$. The coefficients J_n are to be determined.

The variations of gravity with longitude, although ignored in equation (1), can be measured, and the variations of the geopotential with both latitude and longitude, in terms of tesseral harmonics, have been evaluated by analysing observations of satellites with cameras and lasers, and also by Doppler radio tracking, combined with ground-based data such as gravimetric measurements. Two recent examples are the Smithsonian Standard Earth III (ref. 4) and the Goddard Earth Model 4 (ref. 5). The neglect of the longitudinal variations in equation (1) means that U represents

a longitude-averaged potential, and therefore gives a longitude-averaged polar section of the geoid.

The even zonal harmonics, with coefficients $J_2, J_4, J_6 \dots$ are symmetrical about the equator; the odd zonal harmonics, with coefficients $J_3, J_5, J_7 \dots$ are antisymmetrical about the equator. The dots in the previous sentence nicely express the crux of the problem of determining the coefficients: how do you evaluate an infinite set of coefficients from finite data? In practice, the usual method is to analyse the orbital variations in as many satellites as possible, having the most varied available orbits, and to evaluate the coefficients by truncating the series of harmonics when the inclusion of further coefficients seems to bring no advantage.

Table 1 Values of Eight Coefficients			
$10^9 J_3 = -2,531 \pm 7$	$10^9 J_{11} = 159 \pm 16$		
$10^9 J_5 = -246 \pm 9$	$10^9 J_{13} = -131 \pm 22$		
$10^9 J_7 = -326 \pm 11$	$10^9 J_{15} = -26 \pm 24$		
$10^9 J_9 = -94 \pm 12$	$10^9 J_{17} = -258 \pm 19$		

Our recent determination of the odd zonal harmonics³ utilised twenty-seven orbits, at inclinations to the equator ranging between 3° and polar, and we found the most satisfactory least-squares fit to the data was obtained by evaluating eight coefficients, J_3 to J_{17} . Their values are given in Table 1.

The values are determined by fitting the nearly sinusoidal variation in perigee height experienced by the twenty-seven satellites, and the effect of the complete set of harmonics is best characterised by the amplitude β of the oscillation in eccentricity which they produce in a standardised orbit. Figure 1 shows the values of β for an orbit with near-zero eccentricity and semi-major axis $a=7,087$ km ($=R/0.9$).

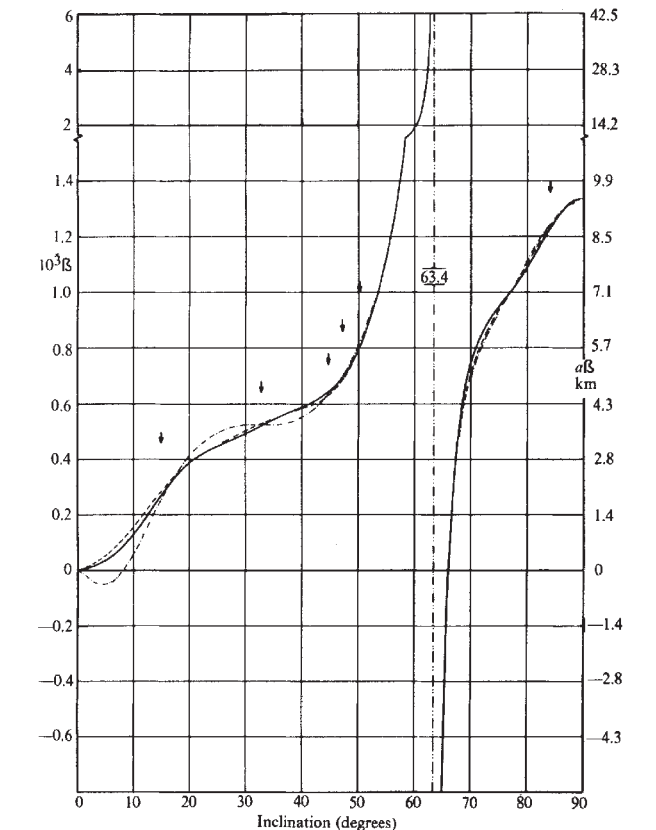


Fig. 1 Variation of β with inclination for $R/a=0.9$ and $e=0$, given by our solution (—), Wagner's⁶ (---) and Kozai's⁷ (— · —). Arrows indicate the inclinations of satellites in all three.

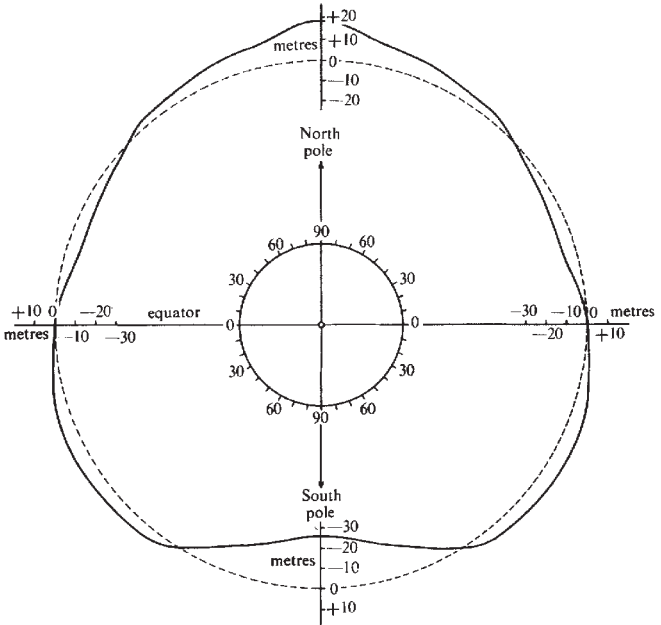


Fig. 2 Height of the meridional geoid section (—) relative to a spheroid of flattening $1/298.25$ (---). (With our odd harmonics and Wagner's⁶ even harmonics.)

Figure 1 also indicates the amplitude of the oscillation in perigee height, $a\beta$, which is nearly independent of a . The diagram gives the value of β for our set of odd zonal harmonics (solid line); for that obtained from twenty-one orbits by Wagner⁶ (broken line); and for that obtained from fourteen orbits by Kozai⁷ (dot-dash line). Our predicted values of $a\beta$ agree with Wagner's to within 200 m except very close to 63.4°, but Kozai's values tend to oscillate about ours, and differ by up to 700 m at some inclinations. There are eight satellites common to all three of these determinations of the odd harmonics, and it is noticeable that the values of β agree well at the inclinations of these satellites, indicated by arrows in Fig. 1. Much of the difference between the three sets no doubt arises from the different individual coefficients evaluated: we chose J_3 to J_{17} ; Wagner chose J_3 to J_{21} ; and Kozai chose J_3 to J_{28} plus J_{35} .

Our previous set of odd zonal harmonics⁸ indicated that the pear-shape tendency of the Earth—the north polar radius minus the south polar radius—amounted to 40 m. The new set of values gives a geoid which is the same to within 1 m over most of the latitude range, but shows larger differences (of up to 2.3 m) at latitudes of 88° to 90°, with the north pole quite literally 'growing a stem', like a pear. Consequently the pear-shape tendency, as defined above, is now greater, 44.7 m instead of 40 m. In presenting a profile of the geoid, it is also necessary to choose a set of even zonal harmonics, and Fig. 2 shows the geoid profile (solid line) relative to a spheroid of flattening $1/298.25$ (broken line) with our set of odd zonal harmonics and Wagner's set of even zonal harmonics⁶. (The use of Kozai's even harmonics⁷ does not alter the profile by more than 0.3 m.) Figure 2 shows that the north polar stem of the pear may be taken as 18.9 m high, whereas the south polar depression is 25.8 m below the spheroid. Since the change from the 1969 values amounted to less than 1 m over most of the latitude range, the profile in Fig. 2 should be accurate to 1 m, although of course it represents an average over all longitudes rather than a specific physical profile.

In conclusion we emphasise that, in spite of the good accuracy of the geoid section, there is still much room for improvement in evaluating the odd zonal harmonics. There are gaps in the coverage in inclination at angles between 6° and 14°, between 16° and 28° and between 33° and 41°. Until these gaps are filled by the launch of suitable satellites,

the validity of the fitting of β in the empty regions will remain open to question.

D. G. KING-HELE
G. E. COOK

Royal Aircraft Establishment,
Farnborough, Hampshire

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¹ Merson, R. H., and King-Hele, D. G., *Nature*, **182**, 640 (1958).

² O'Keefe, J. A., Eckels, A., and Squires, R. K., *Science*, N.Y., **129**, 565 (1959).

³ King-Hele, D. G., and Cook, G. E., *Royal Aircraft Establishment Tech. Rep.*, 73153 (1973).

⁴ Gaposhkin, E. M., Williamson, M. R., Kozai, Y., and Mendes, G., *Smithsonian astrophys. Obs. spec. Rep.*, 380 (1973).

⁵ Lerch, F. J., Wagner, C. A., Putney, B. H., Sandson, M. L., Brown, J. E., Richardson, J. A., and Taylor, W. A., *Goddard Space Flight Center Rep.*, X-553-72-331 (1972).

⁶ Wagner, C. A., *Goddard Space Flight Center Rep.*, X-553-72-341 (1972).

⁷ Kozai, Y., *Smithsonian astrophys. Obs. spec. Rep.*, 381 (1973).

⁸ King-Hele, D. G., Cook, G. E., and Scott, D. W., *Planet. Space Sci.*, **17**, 629 (1969).

Microearthquake Study of the Mid-Atlantic Ridge near 37° N, using Sonobuoys

IN view of the successful use of telemetering radio sonobuoys for detecting and locating microearthquakes in the Gulf of California¹, we carried out a similar survey of the Mid-Atlantic Ridge, in the region near latitude 37° N, during May and June of this year. Our object was to study the level of distribution of seismicity; in particular, we hoped to locate some earthquakes sufficiently accurately that we could associate them with local physiographic features, and draw conclusions about the present tectonic activity of this section of the ridge. Teleseismic study of the mid-ocean ridge suffers from two basic limitations: the epicentral locations are not sufficiently accurate to allow more than a general correlation of seismic activity with the median rift (where present) and with the transform faults offsetting the ridge segments², and it is not generally possible to locate earthquakes of magnitude less than about 4 (ref. 3). Ocean

bottom seismometers have also been used to study ridge seismicity, but the results to date have been fairly limited.

The study described here was undertaken as an ancillary programme on cruise 31 (WHOI TOW I) or RV Knorr (Woods Hole Oceanographic Institution). The main object of this cruise was a detailed survey of the area, using the Scripps Institution of Oceanography/Marine Physical Laboratory's deep-tow instrument package⁵. This, in turn, was part of a continuing intensive study of this section of the Mid-Atlantic Ridge (Project FAMOUS). As a result, the topography is well enough known to allow meaningful correlation with the seismicity.

The technique used was basically that described by Reid *et al.*¹ for the Gulf of California. Expendable sonobuoys⁶ were monitored by onboard recording equipment. The sonobuoy positions were found by firing explosive charges to determine their range from the ship. Whenever possible, we tried to maintain an array of at least three sonobuoys operating, with a spacing of a few kilometres. We obtained fifty-nine epicentral locations, out of 104 earthquakes recorded during 11 d of favourable recording conditions. More than half the locations have an estimated error of 2 km or less.

Figure 1 shows the record of a typical event at a distance of about 30 km. We follow the phase identification and nomenclature of Reid *et al.*¹. The P and S arrivals are the direct compressional and shear waves through the crust, and the T phase is interpreted as the wave travelling through the water layer from the epicentre. At distances less than about 10 km, S and T phases are not normally seen, and the observed phases are P and its reflections in the water column, designated P₁, P₂, and so on.

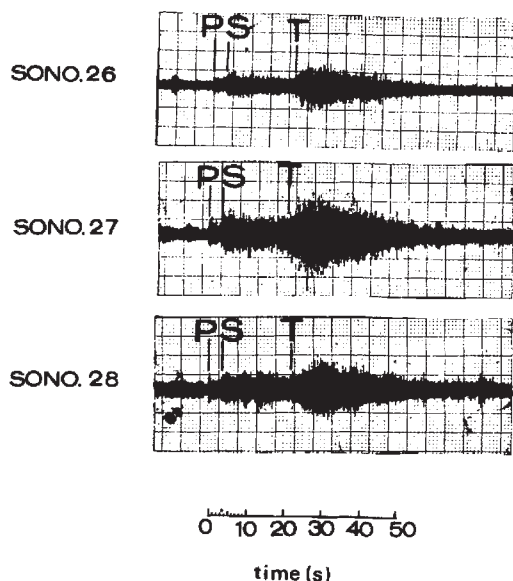


Fig. 1 Recording of a typical earthquake detected on sonobuoys. The epicentral distance is about 30 km. (This is not the original record but a replay off magnetic tape.)

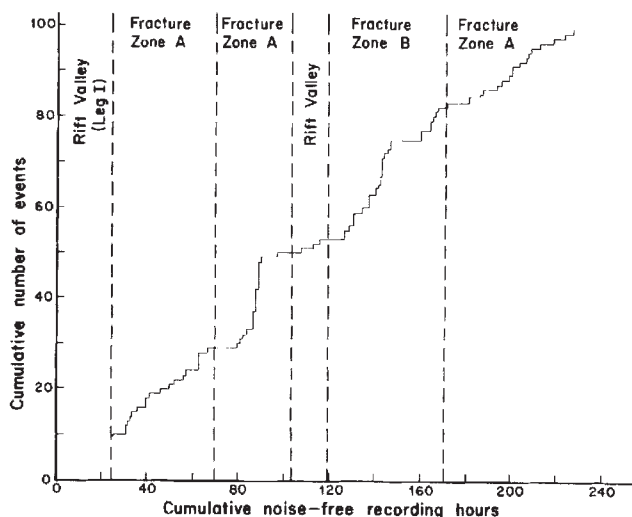


Fig. 2 Cumulative number of earthquakes recorded against cumulative number of low-noise recording hours. The dashed vertical lines indicate the successive sonobuoy arrays.

The results are summarised in Figs 2 and 3. Figure 2 is a plot of cumulative number of earthquakes recorded against cumulative number of low-noise recording hours, and Fig. 3 shows the located epicentres superposed on a bathymetric contour map of the area. As Fig. 3 shows, the study area includes a ridge segment with a well defined median rift, 45 km long and averaging 7 km width. This is terminated by two fracture zones, designated fracture zone A (to the north) and fracture zone B (to the south), which offset the ridge from its continuations. Figure 2 also shows in which part of the area the sonobuoy arrays were placed. On the first leg of the cruise, during which the main survey of the rift took place, the weather was consistently poor, result-

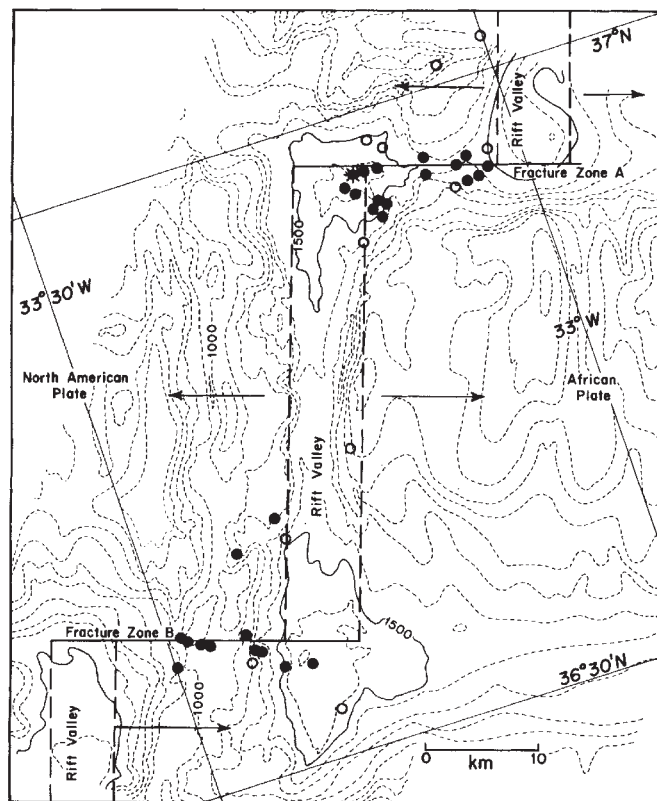


Fig. 3 Map of the best located epicentres, superposed on a bathymetric map of the area. The tectonic interpretation shown is a simplified approximation. ●, Good locations (generally with less than 2 km error); ○, less reliable locations (up to about 5 km error). The star-like symbol at the west end of fracture zone A represents a swarm-type sequence of about seven recorded events.

ing in a noise level too high for effective recording. During the second leg, operations were concentrated on the fracture zones, particularly fracture zone A, and this is reflected in Fig. 2 and in the density of epicentres in Fig. 3.

The seismicity seems to be quite uniform in time (Fig. 2) and the same for both fracture zones. The sample of rift seismicity is not sufficient to allow firm conclusions to be drawn, but it seems lower than the fracture zone seismicity. Figure 2 has been compiled from the raw data, with no corrections for earthquake magnitude or for distance from the array. But we believe Fig. 2 is a fair representation of the seismicity, at least for the fracture zones. We have not attempted any individual magnitude estimates; the detection threshold for a nearby event is about $M=0$, and we estimate most of the earthquakes recorded to be between $M=0$ and $M=1$. In contrast to the Gulf of California or the Galapagos spreading centre, where microearthquake swarms seem to predominate^{1,7} there were only two or three swarm-like sequences⁸ recorded, consisting of up to seven closely spaced events. The microseismicity, at ten events per day, is comparable with that on active sections of the San Andreas Fault⁹, and is consistent with the number of teleseismically detected earthquakes occurring along this section of the Mid-Atlantic Ridge¹⁰.

Figure 3 demonstrates the excellent correlation of the seismicity with the fracture zones deduced from the topography. It clearly shows that most activity is restricted to the section of fracture zone between the rift segments, that is the section where transform faulting¹¹ is occurring. Along fracture zone B, the seismicity appears to delineate a simple transform fault, characterized by a narrow line of epicentres and narrow topographic notch (seen on the deep-tow data but not on the Fig. 3 map). Fracture zone A comprises a

broader region of deformation, with a number of fault scarps and grabens (R. Detrick, personal communication). Most of the computed fracture zone A epicentres, however, lie within 1 km of a straight line, well inside the location uncertainty, which we estimate to be less than 2 km in most cases. This suggests that only one fault plane may be presently active. The active transform faults, as delineated by the seismicity, seem to be narrow and linear, even on a small scale. They are also active over their entire length, even over short time intervals. They show the usual orthogonality to the ridge axis¹².

The junctions of the fracture zones with the median rift are characterized by topographic depressions. The deep-tow study showed that the depression at the junction of fracture zone A with the central rift is bounded on the north by a well defined fault scarp, which we interpret as the active transform fault. This scarp showed a high level of seismic activity, including the only located swarm, and we located one event on this scarp with particularly high accuracy (within 0.5 km). This suggests that active transform faulting continues well into the central rift valley, and hence, that the spreading centre plate boundary (or zone of intrusion), is itself a narrow feature within the central valley.

Because of the small sampling and, we believe, lower seismicity, there are hardly any reliable locations along the rift itself. Such evidence as there is suggests that the seismicity may be concentrated near the edge of the rift, and that there is essentially no activity outside the rift. This would be expected if normal faulting on the rift walls were the dominant source of rift earthquakes¹³. If the low microseismicity of the rift, particularly the low incidence of swarms, is real, then this is in sharp contrast to the Gulf of California and the Galapagos spreading centre, where the spreading segments are highly active^{1,7}. This might indicate less volcanic activity on this slower spreading ridge⁸.

We found it difficult to obtain accurate focal depths in this study, due to array limitations. In one case we found a focal depth of about 1 ± 1 km for a fracture zone A event. Other evidence, particularly the predominant T phase of many events, suggests an equally shallow source for most of the events recorded. In obtaining the epicentre locations, we have generally assumed zero focal depth and a P velocity of 5 km s^{-1} . This should not lead to appreciable errors in most cases. In general, the detailed seismicity and earthquake distribution seem to be in good agreement with current ideas on mid-ocean ridge plate tectonics.

We thank the Chief Scientists of the expedition, Drs B. P. Luyendyk, F. N. Spiess and J. D. Mudie, for accommodating and assisting this work and for allowing us to make use of the deep-tow data, and Dr J. D. Phillips for allowing us to use the bathymetric map of Fig. 3. This research was supported by National Science Foundation grants.

IAN REID

*Institute of Geophysics and
Planetary Physics and
Scripps Institution of Oceanography,
University of California, San Diego,
La Jolla, California 92037*

KEN MACDONALD

*Department of Geology and Geophysics,
Woods Hole Oceanographic Institution,
Woods Hole, Massachusetts 02543
and
Department of Earth and Planetary Sciences,
Massachusetts Institute of Technology,
Cambridge, Massachusetts 02139*

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- ¹ Reid, I., Reichle, M., Brune, J., and Bradner, H., *Geophys. J. R. astr. Soc.* (in the press).
- ² Sykes, L. R., Oliver, J., and Isaacs, B., *The Sea*, 4 (edit. by Maxwell, A. E.), 353 (Interscience, New York, 1971).
- ³ Evernden, J. F., *Bull. seism. Soc. Am.*, **59**, 1365 (1969).
- ⁴ Francis, T. J. G., and Porter, I. T., *Nature*, **240**, 547 (1972).
- ⁵ Spiess, F. N., and Mudie, J. D., *The Sea*, 4 (edit. by Maxwell, A. E.), 205 (Interscience, New York, 1971).
- ⁶ Le Pichon, X., Ewing, J., and Houtz, R. E., *J. geophys. Res.*, **73**, 2597 (1968).
- ⁷ Macdonald, K. C., and Mudie, J. D., *Geophys. J. R. astr. Soc.* (in the press).
- ⁸ Sykes, L. R., *J. geophys. Res.*, **75**, 6598 (1970).
- ⁹ Brune, J. N., and Allen, C. R., *Bull. seism. Soc. Am.*, **57**, 277 (1967).
- ¹⁰ Francis, T. J. G., *Earth planet. Sci. Lett.*, **4**, 39 (1968).
- ¹¹ Wilson, J. T., *Nature*, **207**, 343 (1965).
- ¹² Menard, H. W., and Atwater, T., *Nature*, **219**, 463 (1968).
- ¹³ Sykes, L. R., *J. geophys. Res.*, **72**, 2131 (1967).

Location of Hydrogen Atoms in ADP by Neutron Powder Profile Refinement

NEUTRON diffraction is a powerful tool for the location of light atoms, especially hydrogen, because the neutron scattering power of these atoms is comparable to that of the heavier elements. In particular, the study of hydrogen bonding and its effect on the physical properties of molecular crystals is well suited to neutron diffraction techniques. Unfortunately, because of the relatively low flux of thermal neutrons available, even from the best reactors, rather large single crystals are required, together with expensively long periods of time for data collection. Even when large crystals can be grown, multiple scattering (extinction effects) introduce systematic errors which limit the precision with which atoms can be located. It would be important then, if some way could be found to eliminate the need for large single crystals and long periods for data collection.

The powder diffraction method solves some of these problems, but it is usually thought that neutron powder patterns contain too little information to allow the solution of any but the most simple crystal structures. I argue that provided we abandon the traditional method of structure refinement

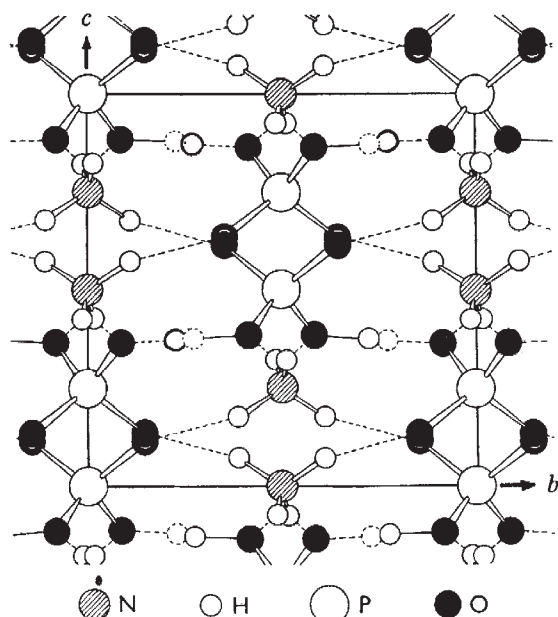


Fig. 1 The [100] projection of the $\text{NH}_4\text{H}_2\text{PO}_4$ structure showing the two positions between which the hydrogen atom linking PO_4 groups can tunnel (after Tenzer *et al.*⁴).

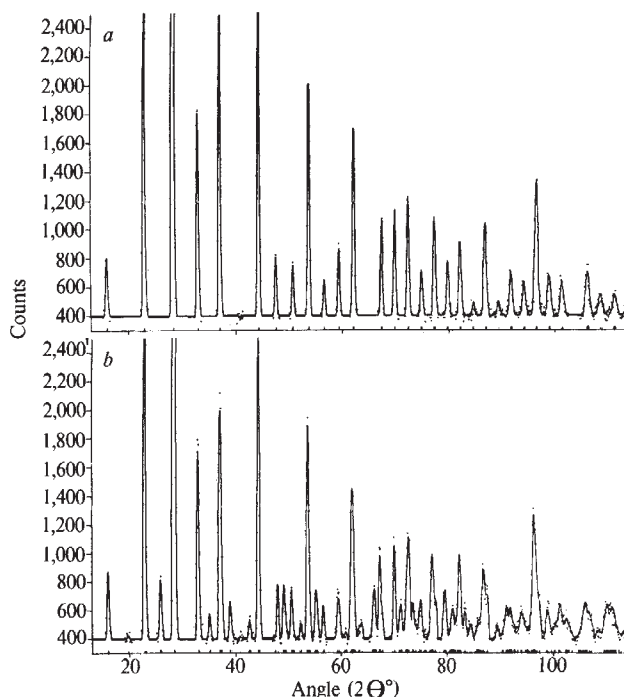


Fig. 2 Observed and calculated powder profiles of deuterated ADP for 1.54 Å neutrons. The positions of the separate Bragg reflexions contributing to these profiles, which were obtained in 22 h on the PANDA diffractometer at Harwell, are marked along the 2θ scattering angle axis. The superlattice lines in the low temperature phase appear because the ordering of the $\text{O}-\text{H} \cdots \text{O}$ hydrogen atoms reduces the symmetry from $I\bar{4}2d$ at room temperature (a) to $\text{P}2_12_12_1$ at 77.4 K (b).

based on the measurement of structure factors, there is much more useful information in a neutron powder pattern than is immediately obvious.

Information is lost, of course, because the crystalline grains in a powder have all orientations; the intensity scattered into a particular direction is the sum of all reflexions having that particular Bragg angle. Because of the finite resolution of a diffractometer, this usually results in a very complicated diffraction pattern from which it is impossible to resolve individual reflexions and to obtain a set of structure factors. Rietveld¹ has shown that in this case it is best to refine the structure to fit directly the observed diffraction pattern, rather than to try to extract the structure factors as an intermediate step. Fortunately, because of the simple radiation spectrum and geometry of a neutron diffractometer, the shape of a single Bragg powder peak is known very precisely for every scattering angle: it is a Gaussian. The positions of all these peaks are fixed by the crystal lattice constants, and so the detailed shape of the entire pattern must be determined solely by the positions of the atom in the crystal lattice; these positions can be refined to obtain the best agreement between the calculated and observed diffraction pattern "profiles".

This profile refinement technique has proved very valuable for the structure refinement of inorganic materials^{1,2}, but now I apply it to more complicated crystals, especially to hydrogen bonded structures. ADP, ammonium dihydrogen phosphate ($\text{NH}_4\text{H}_2\text{PO}_4$) is a rather single example, but the hydrogen bonds are responsible for a very interesting structural transition which occurs at ~ 245 K in the deuterated material. The (H_2) hydrogen atoms have two stable positions, close to one or the other of two oxygen atoms in adjacent PO_4 groups⁴. At room temperature these hydrogen atoms have sufficient energy to tunnel between the two stable positions, and the average occupancy of both positions is $\frac{1}{2}$. When the temperature is lowered the hydrogen atoms are presumably caught in one or the other of these positions, but there is no random

Table 1 Fractional Coordinates of the Deuterium Atoms on the O-H...O Bonds, obtained in this Experiment

	Paraelectric phase $I\bar{4}2d$				Antiferroelectric phase $P2_12_12_1$			
	$a=b=7.502 \text{ \AA}$ $T=22^\circ \text{ C}$	y	$c=7.520 (2) \text{ \AA}^*$ $R=2.8\%$	Occupation	$a=7.507 \text{ \AA}$ $T=77.4 \text{ K}$	$b=7.529 (2) \text{ \AA}^*$	$c=7.445 (2) \text{ \AA}^*$ $R=2.8\%$	Occupation
	x		z		x	y	z	
D1	0.277 (3)	0.144 (1)	0.140 (4)	$\frac{1}{2}$	0.275 (3)	0.144 (3)	0.134 (3)	1
D2	0.223	0.144	0.110	$\frac{1}{2}$				0
D3	-0.144	0.277	-0.140	$\frac{1}{2}$	-0.146 (3)	0.277 (3)	-0.145 (3)	1
D4	-0.144	0.223	-0.110	$\frac{1}{2}$				0

* From observed ratios b/a , c/a : the neutron wavelength $\lambda=1.53 \text{ \AA}$ was not measured precisely.

In the low temperature antiferroelectric phase, these atoms are ordered on one of the two sites over which they are distributed in the room temperature paraelectric phase (see Fig. 3). The R factors are for separated structure factors, and the numbers in parentheses are the standard deviations for the parameters¹.

distribution; there seems to be an ordering of these hydrogen bonds which results in a distortion of the structure from tetragonal ($I\bar{4}2d$) to orthorhombic $P2_12_12_1$. This mechanism is similar to that found by Bacon and Pease³ to be responsible for the ferroelectric transition in KH_2PO_4 , but in the ammonium salt the transition is antiferroelectric. It is important to find the reason for this difference, but experiments on the low temperature structure of $\text{NH}_4\text{H}_2\text{PO}_4$ are difficult: the crystal shatters when passed through the transition because the different domains distort in different directions. This difficulty has held up the solution of the low temperature structure for twenty years.

This experimental problem does not exist for a powdered specimen. In Fig. 2 I show the diffraction pattern obtained in 22 h from 15 g of deuterated ADP, both above (a) and below (b) the transition temperature. The strong "super lattice" reflexions observed in the low temperature phase reflect the lowering of the symmetry which occurs when the deuterium atoms order on one or the other of the sites available to them, thereby distorting the whole structure. The solid lines in these diagrams represent the calculated profiles which best fit the observed points. In both cases, the fit approaches that expected for purely statistical errors in the count. The coordinates and vibrational amplitudes of all of the atoms in both structures have been obtained, but the structural transition can most simply be described if I refer only to the (H_2) hydrogen positions.

In Table 1 I give the coordinates obtained for the two equivalent hydrogen sites in the high temperature phase, when each is occupied for half of the time. In the low temperature phase, I find that the diffraction pattern can be explained if I assume that only one of these alternative positions is occupied. When the occupation numbers for the two positions were refined, starting with $\frac{1}{2}$ for both, the one approached unity while the other approached zero within the limits of uncertainty imposed by the data (1.0 ± 0.1). Figure 3 shows how these positions are taken up to produce a perfectly ordered arrangement of hydrogen bonds, but one in which there is no net dipole moment for the complete cell. This result confirms the ordering proposed by Nagamiya⁵. The PO_4 group at $(\frac{1}{2}, \frac{1}{2}, \frac{1}{2})$, which was equivalent to that at (000) in the high temperature phase, becomes inequivalent in the low temperature phase because of this ordering of hydrogen atoms. These phosphate groups, and the ammonium groups too, are slightly distorted by this hydrogen atom ordering (the ammonium groups are displaced as well), but the resulting dipole moments cancel out over the entire cell and the low temperature structure is antiferroelectric rather than ferroelectric as in KH_2PO_4 .

Finally I note that the profile powder method is a technique for structure refinement: it is not intended to compete with methods such as those based on Fourier techniques for the solution of structures about which very little is known. But with neutron diffraction we are almost always trying to refine the details of a structure which has already been solved approximately by X-ray or other techniques—finding more precise positions for the atoms, especially those of hydrogen and the lighter elements, measuring magnetic moments, nuclear and magnetic disorder, defect structures, atomic vibrational amplitudes and so on. For these structure refinement problems the profile refinement of neutron powder patterns often has many advantages over single crystal method. New designs for neutron powder diffractometers, giving greatly increased resolution and intensity, will allow larger structures to be studied with this technique.

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A. W. HEWAT*

Materials Physics Division,
UKAEA Research Group,
Harwell, Berkshire

Received July 6; revised August 7, 1973.

* Now at Institut Laue-Langevin, BP156 Centre de tri, 38042 Grenoble Cedex.

¹ Rietveld, H. M., *J. appl. Crystallog.*, **2**, 65 (1969).

² Hewat, A. W., *J. Phys. C.*, **6** (in the press).

³ Bacon, G. E., and Pease, R. S., *Proc. R. Soc.*, **A230**, 359 (1955).

⁴ Tenzer, L., Frazer, B. C., and Pepinsky, Ray, *Acta Crystallog.*, **11**, 505 (1958).

⁵ Nagamiya, T., *Progr. theoret. Phys. Japan*, **7**, 275 (1952).

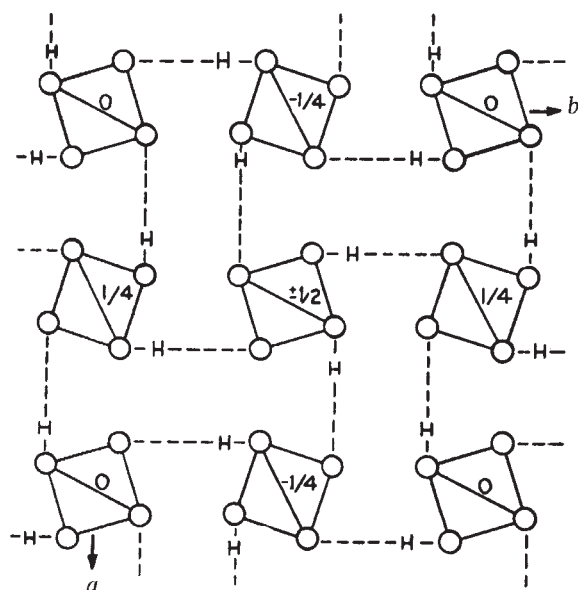


Fig. 3 Ordering of the deuterium atoms (H) on the hydrogen bonds between the phosphate groups, as proposed by Nagamiya⁵ and verified in the present experiment.

BIOLOGICAL SCIENCES

Suppression of Mouse Antibody Producing Spleen Cells by Various Interferon Preparations

POLYRIBONOSINIC acid: polyribocytidylic acid [poly (rI)-poly (rC)], endotoxin, polycarboxylate plastics or polysaccharides, and other polyanions induce interferon production (see reviews ref. 1 and 2). They also enhance antibody production^{3,4}, cell-mediated immunity⁵, and reticulo-endothelial activity^{4,6}. It is not yet clear whether or not any of these latter effects are mediated through the action of interferon.

Recently, mouse interferon preparations from several different sources have been shown to enhance phagocytic activity in peritoneal macrophages cultivated *in vitro*⁷. Also, many reports have shown antitumour activity in interferon preparations both *in vivo* and in cell culture (see review ref. 2).

Attempts have been made to measure the effects of interferon preparations on *in vivo* antibody production, but these studies demonstrated either no effect⁸ or variable effects, including modest stimulation or inhibition⁹. Large amounts of interferon are required to simulate *in vivo* the circulating interferon levels produced by inducers such as poly (rI)-poly (rC), and past work has been restricted to interferon levels considerably lower than those obtained with inducers. Our studies were conducted with potent interferons, and measured the effects of a variety of interferon preparations on the number of mouse spleen cells producing antibody to sheep red blood cells (SRBC) during primary immunization. Interferon was administered in several large doses over 3 h, giving a reasonable simulation of both the levels and the time course of circulating interferon following poly (rI)-poly (rC) administration.

Four different interferon preparations, stimulated by Newcastle Disease virus (NDV), were used: (1) pressure dialysed L₉₂₉ cell supernatant, (2) pressure dialysed C243-3C cell¹⁰ supernatant, (3) mouse serum, and (4) L cell interferon which was purified by affinity chromatography using anti-mouse interferon antibody to a purity of 1.96×10^8 units mg⁻¹ protein¹¹.

When 100 μ g of poly (rI) poly (rC) was given either 2 d or 4 d before antigen, a two-fold increase in the number of antibody producing spleen cells assayed 6 d after antigen administration occurred (Table 1). To determine the amount of interferon necessary to successfully simulate the circulating interferon levels induced by poly (rI)-poly (rC), two different doses of interferon were administered, sera were taken at various times, and then the interferon titres compared to those achieved after intravenous (i.v.) administration of 100 μ g of poly (rI) poly (rC). In one case approximately 1.8×10^5 units of L₉₂₉ cell interferon were administered i.v. in four divided doses over a 4 h period, in the other, approximately 4.2×10^4 units were similarly given. As can be seen from the results plotted in Fig. 1, the larger dosage schedule resulted in serum interferon levels approximately equal to that achieved by poly (rI)-poly (rC) while the lower dosage level did not achieve adequate serum levels to simulate the interferon production of the dose of poly (rI)-poly (rC) necessary to simulate an increase in the number of antibody producing spleen cells.

When 1.8×10^5 units of L₉₂₉ cell interferon were thus given 2 d before antigen (SRBC), at a time when poly (rI)-poly (rC) caused a marked enhancement of the number of antibody producing spleen cells as assayed 6 d after antigen administration (Table 1), we were surprised to note a sharp depression in the number of antibody-producing spleen cells to 16% of that in uninjected control animals.

This experiment was repeated several times with different interferon preparations. As can be seen in Fig. 2 and Table 1, as the interferon titre increased, the degree of depression increased, giving a sigmoidally shaped dose response curve characteristic of the antiviral action of interferon. Doses of over 1×10^5 units of interferon gave marked depression (16% of control), while doses between 5×10^4 units and 1×10^5 units showed an intermediate degree of depression with a greater degree of variability. Doses of less than 5×10^4 units showed no measurable depression.

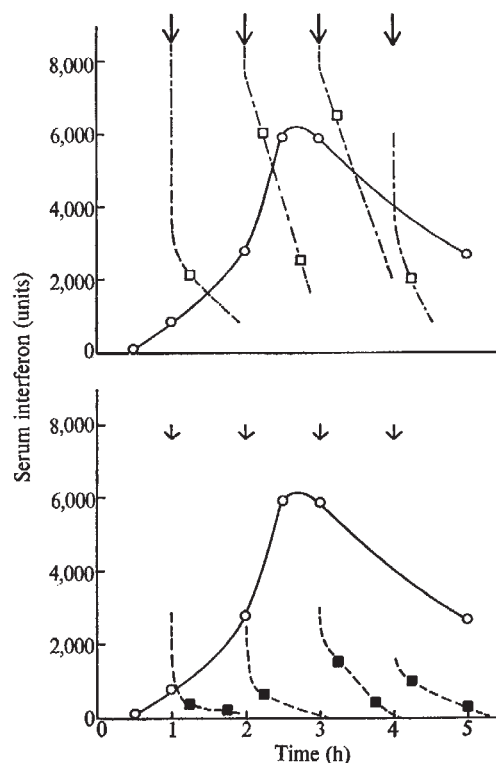


Fig. 1 Comparison of serum interferon attained after i.v. injection of various doses of interferon with that attained after induction by poly (rI)-poly (rC). Interferon titres were measured with a plaque reduction assay on mouse L₉₂₉-cell monolayers employing bovine vesicular stomatitis virus (VSV), Indiana strain, as a challenge virus²⁰. The interferon titre corresponded to the reciprocal of the highest dilution of sample which reduced virus plaque formation by 50%. Titration of the 6,000 unit mouse interferon standard distributed by the National Institutes of Health revealed a unitage of 2,490 units per vial in this assay. Serum levels of interferon attained in 6-week-old Swiss-Webster mice after intravenous administration of 100 μ g of poly (rI)-poly (rC) at time 0 (○—○). Serum levels of interferon attained after intravenous administration of L₉₂₉ cell interferon (titre 5.2×10^4 units), 1.0 ml at 1, 2, and 3 h and 0.5 ml at 4 h (□—□), and after L₉₂₉ cell interferon (titre 1.2×10^4 units) at the same times and amounts (■—■). Clearance curves are extrapolated to expected concentrations at zero time after injection.

To test whether volume of injection or the presence of other non-interferon substances may be responsible for this effect, two preparations of supernatant from uninduced L₉₂₉ cell monolayers and one preparation of mouse serum from uninduced mice were inoculated in the same volumes as were the interferon preparations with no significant deviation from control values (Fig. 2). These preparations were in all respects processed and concentrated similarly to their corresponding interferon preparations, and all titred at less than 10 units of interferon. In addition, a portion of one batch of C243-3C cell interferon preparation was exposed to trypsin (0.25 mg ml⁻¹) for 1 h at 37° C and another heated to 56° C for 1 h. These preparations along with an untreated portion were inoculated into a series of mice. The untreated portion showed the usual immuno-

Table 1 Effect of Various Interferon Preparations on Antibody Producing Cells

Substances	Dose	Day of administration relative to antigen	Antibody producing cells* (% of mean control level)		No. of animals	Significance of difference from control
			Mean	Range of $\pm$ s.d.		
Poly (rI)·poly (rC)	100 μ g	2 d before	209%	175% to 251%	10	$P < 0.001$
	100 μ g	4 d before	170%	137% to 210%	7	$P < 0.05$
Interferon, all types†	$> 1 \times 10^5$ units	2 d before	16%	12% to 20%	9	$P < 0.001$
	4×10^4 units					
	to 1×10^5 units	‡	76%	62% to 95%	8	$P < 0.11$
Affinity chromatography purified interferon	1.5×10^5 units	2 d before	19%	12% to 29%	2	$P < 0.001$
Human leukocyte interferon	1×10^5 units	2 d before	86%	56% to 131%	2	(NS)
C243-3C cell interferon						
Untreated	1.5×10^5 units	2 d before	17%	13% to 22%	3	$P < 0.001$
56° C for 1 h	2.5×10^4 units	2 d before	90%	62% to 132%	3	(NS)
Trypsin exposed§	$< 10^2$ units	2 d before	139%	84% to 233%	1	(NS)

* Statistical analysis of the results was carried out by linear regression analysis on the natural logarithms of the observed data, assuming orthogonality between any day-to-day variation in the baseline and experimental effects. The geometric (logarithmic) mean $\pm$ s.d. is given as a percentage of the mean control value. In the computer program used for this analysis, the reported s.d. and confidence limits of the experimental groups are expanded to include the s.d. of the mean control value. Duplicate assays were conducted on each spleen.

† Two animals received L₉₂₉ cell interferon, six C243-3C cell interferon, and one mouse serum interferon.

‡ Five animals 2 d before, one animal 3 d before, and two animals 4 d before.

§ 25 mg ml⁻¹ for 1 h at 37° C.

|| Residual titre after treatment.

(NS) Not significant.

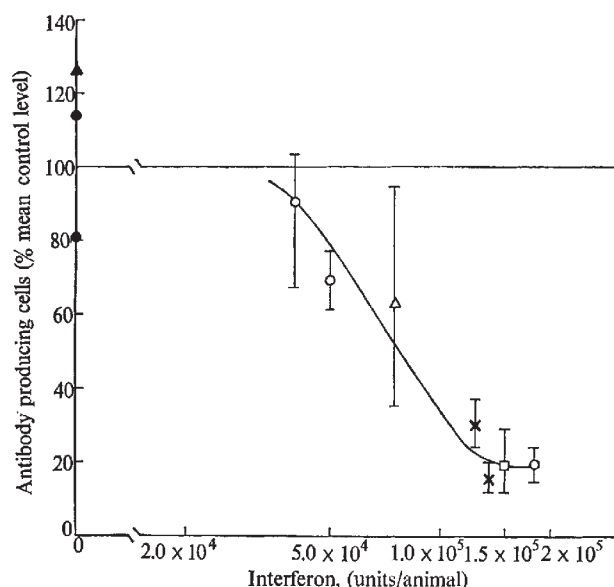


Fig. 2 Relationship of interferon dose and number of antibody producing spleen cells. Animals were sensitized to SRBC antigen by intravenous administration of 2×10^7 SRBC in 0.5 ml saline. Antibody-producing capability was measured by the number of spleen cells producing antibody to SRBC 6 d after antigen administration. Antibody-producing cells were assayed by the plaque technique²¹ as extended for use on microscope slides²² with modifications as previously described⁴. Various doses of interferon were administered 2 d before administration of antigen (SRBC). The number of nucleated spleen cells producing antibody to SRBC was assayed 6 d after antigen administration and expressed as a percentage of the number of antibody producing spleen cells in uninjected controls. All determinations shown represent the mean in at least two animals. Ranges shown are $\pm$ s.d. Two uninduced control preparations containing less than 10 units of interferon were also used. One consisted of pressure dialysed supernatant of L₉₂₉ cell tissue culture medium (●), and the other of serum from untreated mice (▲). Interferon preparations from four sources were used, all induced with NDV. One from L₉₂₉ cells and pressure dialysed 5 to 50-fold (○) (ref. 18), one from C243-3C cells and pressure dialysed 20-fold (×) (ref. 10), one from mouse serum 10 h after injection of NDV (△), and one purified on an anti-interferon affinity chromatography column (□) (ref. 11). Five days of pH 2 treatment was used to inactivate NDV in all interferon preparations and no residual interfering activity as titred in human neonatal fibroblasts was noted in any preparation.

depressant activity (17% of control), while the inactivated preparations showed only activity consistent with their residual antiviral activity (Table 1). To check the species specificity of the depressant activity, 1×10^5 units of human leukocyte interferon were administered to mice with the result that no significant decrease in the number of antibody producing spleen cells was observed (Table 1).

At first the differing effects of interferon inducers such as poly (rI)·poly (rC), which increases the number of antibody producing spleen cells, and interferon preparations which decrease the number of antibody producing spleen cells, seem paradoxical. Recent findings, however, do provide a possible explanation; in addition to inhibition of multiplication of tumour cells, interferon preparations inhibit the multiplication of tissue culture cells in both continuous cell lines^{12,13} and primary cell cultures^{14,15}. Also, interferon preparations have been reported to inhibit DNA synthesis in mouse lymphocyte suspensions stimulated by phytohaemagglutinin¹⁶, and to inhibit proliferation of transferred spleen cells and the ability of these transferred lymphocytes to become sensitized to host alloantigens as assayed by an *in vitro* cytotoxic assay system¹⁷. In view of these findings that interferon depresses cell multiplication, inhibits DNA synthesis in lymphocytes, and otherwise blocks T lymphocyte function, it is not surprising that we should now find that interferon inhibits B lymphocyte expression in response to primary antigen stimulation.

Our results may form grounds for concern with regard to the use of interferon preparations in the treatment of viral infections in humans. It should be noted, however, that circulating interferon titres of the level required to produce these effects are produced transiently during a variety of systemic viral infections in the mouse. Also, concerning the possible clinical usefulness of exogenous interferon, it is important to compare the doses which we find to inhibit antibody producing cells in the spleen with that required to prevent various viral infections. The immunosuppressive doses are approximately 10 to 100-fold greater than that required to prevent a lethal cytotoxic virus infection¹⁸ or virus induced neoplasm¹⁹ in the same species. Previous observation⁹ of an immunosuppressive effect at one twentieth the dosage required in our study could not be confirmed, and further study is needed to elucidate the discrepancy between these two results.

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THOMAS J. CHESTER

Division of Infectious Diseases,
Department of Medicine,
Stanford University School of Medicine,
Stanford, California 94305

KURT PAUCKER

Department of Microbiology,
Medical College of Pennsylvania,
Philadelphia, Pennsylvania 19129

THOMAS C. MERIGAN

Division of Infectious Diseases,
Department of Medicine,
Stanford University School of Medicine,
Stanford, California 95305

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- ¹ De Clercq, E., and Merigan, T. C., *Archs intern. Med.*, **126**, 94 (1970).
- ² Rodgers, R., and Merigan, T. C., *CRC Critical Reviews in Clinical Laboratory Sciences* (June, 1972).
- ³ Braun, W., and Nakano, M., *Science, N. Y.*, **157**, 819 (1967).
- ⁴ Chester, T. J., De Clercq, E., and Merigan, T. C., *Infection and Immunity*, **3**, 516 (1971).
- ⁵ Cantor, H., Asofsky, R., and Levy, H. B., *J. Immun.*, **104**, 1035 (1970).
- ⁶ Regelson, W., and Munson, A. E., *Ann. NY Acad. Sci.*, **173**, 831 (1970).
- ⁷ Huang, K., Donahoe, R. M., Gordon, F. B., and Dressler, H. R., *Infection and Immunity*, **4**, 581 (1971).
- ⁸ Anderson, S. G., *Aust. J. exp. Biol. med. Sci.*, **43**, 345 (1965).
- ⁹ Braun, W., and Levy, H. B., *Proc. Soc. exp. Med. Biol.*, **141**, 769 (1972).
- ¹⁰ Oie, H., Gazdar, A., Buckler, C. E., and Baron, S., *J. gen. Virol.*, **17**, 107 (1972).
- ¹¹ Ogburn, C. A., Berg, K., and Paucker, K., *Abst. Ann. Meeting Amer. Soc. Microbiol.*, **73**, 239 (1973).
- ¹² Lee, S. H. S., O'Shaughnessy, M. V., and Rozee, K. R., *Proc. Soc. exp. Biol. Med.*, **139**, 1438 (1972).
- ¹³ Paucker, K., Cantell, K., and Henle, W., *Virology*, **17**, 324 (1962).
- ¹⁴ Lindahl, P., Leary, P., and Gresser, I., *Proc. Soc. exp. Biol. Med.*, **138**, 1044 (1971).
- ¹⁵ McNeill, T. A., Fleming, W. A., and McCance, D. J., *Immunology*, **22**, 711 (1972).
- ¹⁶ Lindahl, P., Leary, P., and Gresser, I., *Nature new Biol.*, **237**, 120 (1972).
- ¹⁷ Cerottini, J. C., Brunner, K. T., Lindahl, P., and Gresser, I., *Nature new Biol.*, **242**, 152 (1973).
- ¹⁸ De Clercq, E., Nuwér, M. R., and Merigan, T. C., *J. clin. Invest.*, **49**, 1565 (1970).
- ¹⁹ De Clercq, E., and De Somer, P., *J. natn. Cancer Inst. U.S.A.*, **6**, 1345 (1971).
- ²⁰ Merigan, T. C., and Finkelstein, M. S., *Virology*, **35**, 363 (1968).
- ²¹ Jerne, N. K., Nordin, A. A., and Henry, C., in *Cell Bound Antibodies* (edit. by Amos, B., and Koprowski, H.), **109** (Wistar Institute Press, Philadelphia, 1963).
- ²² Mishell, R. I., and Dutton, R. W., *J. exp. Med.*, **126**, 523 (1967).

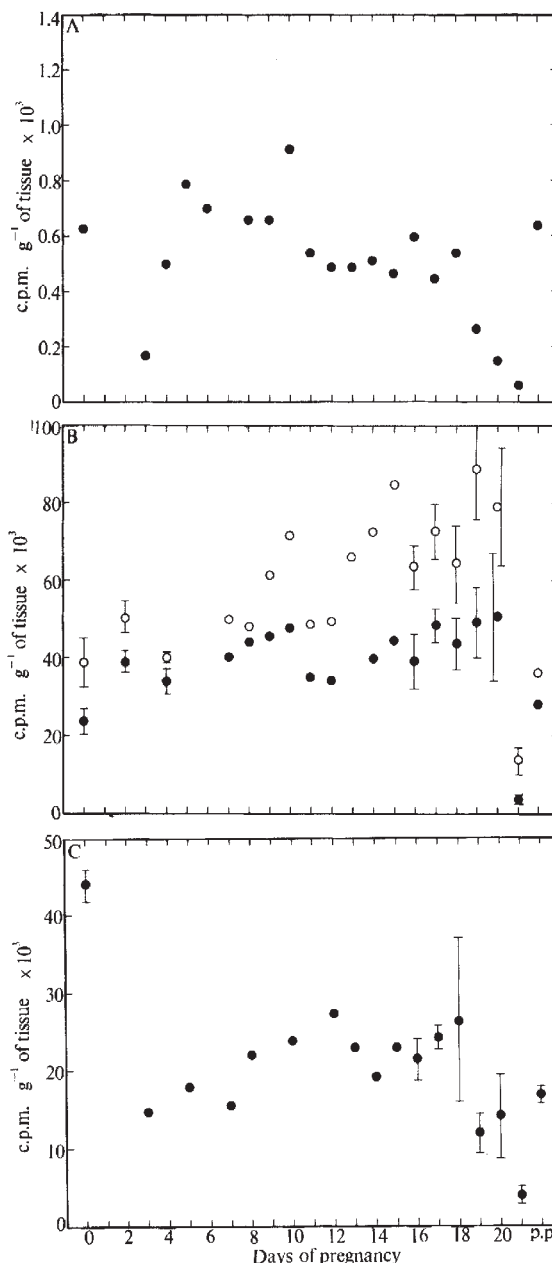


Fig. 1 Incorporation of (A) ^3H -uridine (28 Ci mmol^{-1}), (B) ^3H -cytidine (6 Ci mmol^{-1}), and (C) ^3H -orotic acid (11 Ci mmol^{-1}) into maternal liver 2 h after injection. Bars represent standard error of mean. 0 d pregnancy indicates studies on young male rats. p.p., 24 h post-partum; $\circ$, acid-soluble counts; $\bullet$, acid-insoluble counts.

Changes in the Incorporation of Different RNA Precursors into the Maternal Liver during Pregnancy

DIFFERENT RNA precursors have been utilized to label foetal and maternal RNA *in vivo* with varying results^{1,2}. Recently ^3H -cytidine has been shown to effect the highest level of radioactive incorporation into both the maternal and foetal RNA³. The work reported here was undertaken to compare the incorporation of three separate RNA precursors into the rat maternal liver serially throughout gestation; thus, differences in incorporation that may develop with pregnancy among the individual precursors can be compared directly.

Dated pregnant Sprague-Dawley rats (Zivic Miller, Pittsburgh) weighing 200–420 g were used. Two hours after

intraperitoneal injection of isotopes ($10 \mu\text{Ci}$ per 100 g of body weight), the animals were killed by cervical dislocation. A 2.0 g sample of the maternal liver was homogenised in 9 volumes of cold 0.1 M sodium acetate buffer, pH 5.0, using a Potter-Elvehjem homogeniser. One-tenth volume of 50% trichloroacetic acid was added, stirred and centrifuged. The precipitate was washed twice with 5% trichloroacetic acid. A 0.5 ml sample of the pooled washings and the supernatant was placed in 10.0 ml of Bray's solution to measure the level of radioactivity using a liquid scintillation counter. The precipitate was hydrolysed for 15 min in 10 ml of 10% trichloroacetic acid in a boiling water bath, cooled and centrifuged at $10,000g$ for 10 min. Using 0.5 ml from the resulting supernatant the radioactivity of the acid-insoluble counts was determined in Bray's solution.

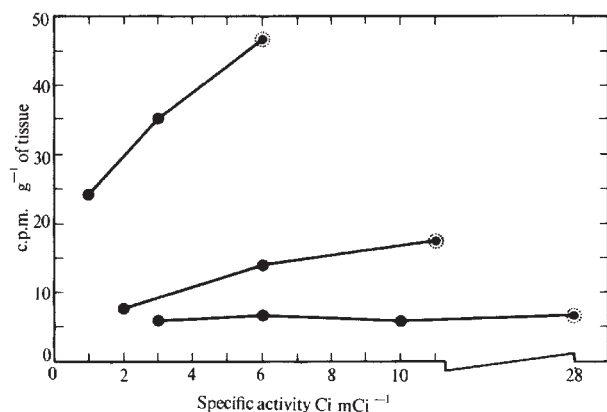


Fig. 2 Incorporation of ^3H -uridine (lower curve), ^3H -cytidine (upper curve) and ^3H -orotic acid (middle curve) into the maternal liver of 16-d pregnant rats with varying specific activities. The experiments were conducted as described in Fig. 1. The total volume of the precursor was kept constant through the study. $\circ$, Represents the specific activity used in experiments shown in Fig. 1.

The 2 h post-injection period was selected since ^3H -cytidine reached maximum incorporation at this time in accordance with a previous study³; whereas ^3H -uridine yielded a consistent and steady level of radioactive incorporation during the same time. The identical 2-h interval was maintained with ^3H -orotic acid only for purposes of comparison since ^3H -orotic acid continued to be incorporated for a longer time.

Figure 1 clearly shows the marked differences in the incorporation of isotopically labelled uridine, cytidine and orotic acid. The non-pregnant rats in this series designated as 0 d represent male rats (170–190 g) because of the wide variations observed from using virgin rats, especially with ^3H -uridine. Additional experiments performed on animals 48 to 72 h after delivery yielded results similar to those noted in the 24 h post-partum group shown in Fig. 1. Thus, the post-partum values may be most representative of the non-pregnant state for comparison with pregnancy. Every point in the figure represents an average of two to three separate animal experiments; when considerable scatter of values was obtained, especially towards the end of pregnancy, an additional six to eight animals were studied to determine the standard error of the mean. Note that early in pregnancy, uridine displayed decreased incorporation while cytidine and orotic acid had values comparable with the post-partum group. Although the orotic acid was incorporated to an unusually high degree among the male rats, cytidine is the most effective during pregnancy, confirming an earlier report³. Uridine was the least efficient precursor in both the pregnant and non-pregnant states, and further experiments revealed no changes in uridine

kinase in the maternal liver during pregnancy. The acid-soluble counts, although graphed only with the cytidine studies, in all cases mirrored the changes observed in the acid-insoluble counts, but the actual values were slightly higher. However, the most dramatic and consistent result was the sharp decrease in the incorporation of all precursors on the twenty-first day of pregnancy, just before parturition. Within 24 h post-partum the level of incorporation returned to earlier pregnant or non-pregnant values.

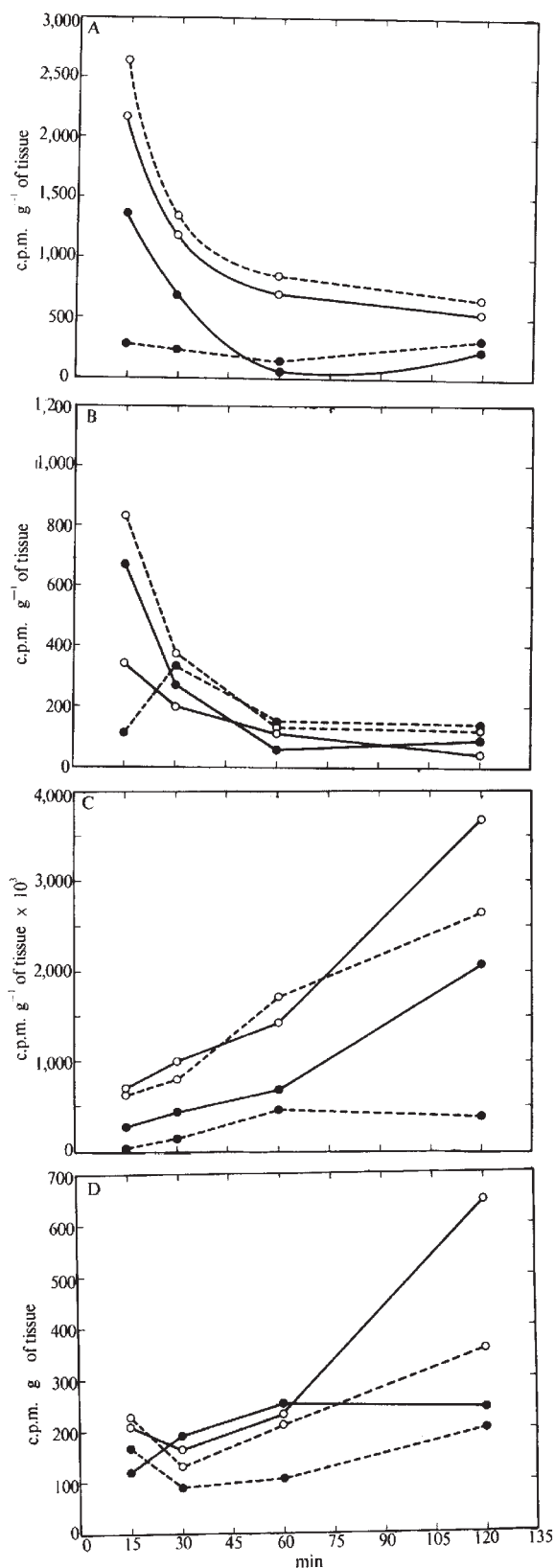


Fig. 3 The incorporation of ^3H -cytidine and ^3H -uridine into the nucleus and cytoplasm of maternal liver during various stages of pregnancy. A and C, Nuclear acid-insoluble counts (uridine); B and D, cytoplasmic acid-insoluble counts (cytidine). Non-pregnant rats represent young male animals. At specific times after intraperitoneal injection of isotopes (10 μCi per 100 g of body weight), the animals were killed. From 4.0 g of liver, 25% homogenate was prepared with 0.1 M sodium acetate buffer, pH 5.0, using Tri-R Homogenizer for ten strokes at 1,200 r.p.m. The homogenate was filtered through four layers of cheese cloth and the nuclear fraction was isolated as a pellet by centrifugation at 700g for 20 min. The nuclear and cytoplasmic preparations were treated with an equal volume of 10% trichloroacetic acid and washed once with 5% trichloroacetic acid. The resulting precipitates were treated and counted as acid-insoluble radioactive counts as described earlier in Fig. 1. $\circ$ — $\circ$, 8 d; $\circ$ — $\circ$, 18 d; $\bullet$ — $\bullet$, 21 d; $\bullet$ — $\bullet$, non-pregnant.

In approximately a quarter of the rats studied, an additional procedure included measurement of radioactivity in the blood. One and a half hours after injection of the labelled precursor, the animals were sedated by intramuscular injection of sodium pentobarbital (30 mg kg⁻¹). Rats were anaesthetised in an ether chamber just before death 2 h after injection and 3.0 ml of blood was obtained by a direct cardiac tap using a 20 gauge needle and a heparinised syringe. Aliquots of 0.1 ml of plasma were placed in 10.0 ml of Bray's solution to determine radioactivity. The maternal liver was homogenised and prepared as described earlier. The level of blood radioactivity measured in the plasma was relatively constant throughout pregnant and non-pregnant animals with only a slight decrease at 21 d. With orotic acid and uridine the radioactivity measured 40,000 to 55,000 c.p.m. ml⁻¹ of plasma; whereas, cytidine showed only 9,000 to 11,000 c.p.m. ml⁻¹ of plasma.

Figure 2 illustrates the differences in incorporation of 16-d pregnant rats using precursors with varying specific activities. Orotic acid showed a slight rise in radioactivity with increasing specific activities, but cytidine exhibited a marked increase. In direct contrast, uridine showed no change with even greater alterations in specific activities suggesting a rapid saturation of its system. Although the actual measured radioactive counts were considerably lower, a similar pattern of response was observed in 21-d pregnant rats with all three precursors.

Figure 3 demonstrates the marked differences in the incorporation of ³H-uridine and ³H-cytidine into the nuclear and cytoplasmic portions of the liver during various stages of pregnancy. Although the actual amount of radioactive labelling is relatively small by comparison, ³H-uridine is incorporated rapidly into both the nuclear and cytoplasmic RNA and then falls off within 30 min. The 21-d pregnant rat shows poor uptake of radioactivity throughout the 2 h period of observation. The incorporation of ³H-cytidine differs in that the isotopic incorporation in both the nucleus and cytoplasm increases during the 2 h period, in a manner similar to that observed using labelled orotic acid. The 8 and 18-d pregnant rats reveal enhanced labelling compared with the non-pregnant rat, but the 21-d pregnant rat displays

a markedly depressed level of incorporation as noted in the ³H-uridine experiments.

To exclude the possible effects of changes in the intra-peritoneal absorption of isotopes, the three labelled precursors were administered intravenously through the tail vein to another group of 16-d and 21-d pregnant animals. Again a comparable labelling of the 16-d pregnant and the depressed incorporation of the 21-d pregnant rats were noted as the results show in Fig. 1.

Table 1 summarises our studies on the nuclear, nucleolar and extranucleolar DNA-dependent RNA polymerases from the livers of 16-d and 21-d pregnant rats. The results indicate that there is no drastic decrease in the specific activity of DNA-dependent RNA polymerases in the 21-d pregnant rat in spite of the marked depression in the incorporation of RNA precursors. There is a measurable drop only in the extranucleolar DNA-dependent RNA polymerase.

The specific causes for the changes in the incorporation of the various RNA precursors during pregnancy remains unresolved although endocrine influences must play a dominant role. The work reported here, however, clearly indicates that pregnancy and the duration of pregnancy have profound effects on the degree of RNA incorporation with different RNA precursors.

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T. TERRY HAYASHI
KATHLEEN MACFARLANE

Department of Obstetrics and Gynecology,
University of Pittsburgh School of Medicine,
Pittsburgh, Pennsylvania 15213

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- ¹ Bresnick, E., Lanclos, K., and Gonzales, E., *Biochim. biophys. Acta*, **108**, 568 (1965).
- ² Hayashi, T. T., Shin, D. H., and Wiand, S., *Am. J. Obstet. Gynec.*, **105**, 1261 (1969).
- ³ Larkin, L. H., and Stevens, A. R., *Nature new Biol.*, **235**, 107 (1972).
- ⁴ Widnell, C. C., and Tata, J. R., *Biochem. J.*, **92**, 313 (1964).
- ⁵ Yu, F. L., and Feigelson, P., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2177 (1971).
- ⁶ Weiss, S. B., *Proc. natn. Acad. Sci. U.S.A.*, **46**, 1020 (1960).
- ⁷ Widnell, C. C., and Tata, J. R., *Biochim. biophys. Acta*, **123**, 478 (1966).
- ⁸ Roeder, R. G., and Rutter, W. J., *Proc. natn. Acad. Sci. U.S.A.*, **65**, 675 (1970).
- ⁹ Fleck, A., and Munro, H. N., *Biochim. biophys. Acta*, **55**, 571 (1962).

Table 1 Comparison of Specific Activity of Liver RNA Polymerases in 16-d and 21-d Pregnant Rats

	16-d pregnant rats	21-d pregnant rats
	Mg ²⁺ -activated	
Nuclear	131	253
Nucleolar	158	161
Extranucleolar	2,199	1,306
	Mn ²⁺ (NH ₄) ₂ SO ₄ -activated	
Nuclear	116	220
Nucleolar	217	165
Extranucleolar	2,716	1,623

Nuclei were isolated from liver homogenate according to Widnell and Tata⁴, and nucleolar and extranucleolar (nucleoplasmic) suspensions were prepared as described by Yu and Feigelson⁵. The RNA polymerase assay was essentially the method of Weiss⁶. The reaction mixture contained 50 μmol of Tris-HCl buffer, pH 8.5; 2.5 μmol of magnesium chloride, 3 μmol of sodium fluoride and 10 μmol of cysteine; 0.3 μmol each of GTP, CTP and ATP and 0.015 μmol of UTP; 2.0 μCi of 5-³H-UTP (Schwarz-Mann, 14.0 Ci mmol⁻¹) and 0.1 ml of enzyme suspension. For the ammonium sulphate assay 2.0 μmol of MnCl₂ and 0.2 mmol of (NH₄)₂SO₄ were used instead of MgCl₂ and the pH of the buffer was 7.5 (ref. 7). After incubation for 15 min at 37° C the reactions were stopped, adding 0.10 M sodium pyrophosphate containing RNA and bovine serum albumin⁸. The RNA was collected on Whatman GF C filters and radioactivity measured with liquid scintillation counter using 10.0 ml Bray's solution. RNA determinations were done according to Fleck and Munro⁹. Values represent an average of three determinations and indicate pmol of UMP incorporation per mg DNA.

Slightly Deleterious Mutant Substitutions in Evolution

RECENT advances in molecular genetics have had a great deal of influence on evolutionary theory, and in particular, the neutral mutation-random drift hypothesis of molecular evolution^{1,2} has stimulated much interest. The concept of neutral mutant substitution in the population by random genetic drift can be extended to include random fixation of very slightly deleterious mutations which have more chance of being selected against than of being selected for^{3,4}. If this class of mutant substitution is important, we can predict that the evolution is rapid in small populations or at the time of speciation⁵. Here I shall organize the observed facts which indicate that this class is in fact important.

Fitch and Margoliash⁶ have shown that the frequency of double fixation of mutants in a leg of their cytochrome c phylogenetic tree is higher than predicted from chance. The

most plausible interpretation is that the first mutant is slightly deleterious and that the second compensates for the first. This interpretation makes Fitch's concept of the covarion⁷ most easily understood. If the first mutant disturbs very slightly the normal function of the molecule, there are probably many ways of compensating, and so the first mutant provides a new possibility for evolutionary change. This is considered to correspond to Fitch's observation of unstable covarions⁷.

The evolution of tRNA follows the same pattern. It is known that evolution is more rapid in the paired region than in the non-paired region of tRNA⁸. In most tRNAs, the paired region is almost exactly paired, therefore there must have been coupled substitutions in evolution as first mentioned by Jukes⁹. Dayhoff and McLaughlin⁸ state "this could be explained by an oscillatory sequence of evolutionary events, involving first a single base change that weakens the bonding, followed by a second change which restores the hydrogen-bond pairing". It is hard to conclude from this that each sequence has evolved so as to fit better into its environment. It indicates rather that the first substitution is slightly deleterious. This idea can be extended to other protein molecules, particularly if the polypeptide-folding mechanisms are important, as discussed by Barnard *et al.*¹⁰. Any amino acid substitution of a well organized molecule is likely to disturb the organization, but once it spreads in the population by random drift, it opens the way for further evolutionary change.

This hypothesis is compatible with a different point of view, that is, frequency distribution of multiple alleles detected by electrophoresis. Several investigators have been trying to prove or disprove the neutral theory of protein polymorphisms by looking at the pattern of allelic distribution^{11,12}. In such attempts, it is usually assumed that every mutant appearing in the population is one not pre-existing, that is to say, the so called "infinite allele model" of Kimura and Crow¹³ has been used extensively. The convenient statistics often used in the analysis are the effective number of alleles (n_e ; reciprocal of the sum of squares of allelic frequencies) and the actual number of alleles observed (n_a). Recently, Yamazaki and Maruyama¹⁴ made an extensive compilation and statistical analysis of published data on enzyme polymorphisms. In particular, they showed that the average ratio of the effective to the actual number of alleles (n_e/n_a) from the observed enzyme polymorphisms agrees well with the neutral hypothesis according to Kimura and Crow's infinite allele model. Here the ratio, n_e/n_a , represents a sort of evenness of allele distribution and, the higher the ratio, the more uniform the distribution expected. But this model seems to be inappropriate for electrophoretically detectable polymorphisms, for this method can only detect a difference in the electric charges of molecules as discrete bands on the gels. In fact Bulmer¹⁵ noticed non-random arrangements of electrophoretic bands of common and rare alleles. In view of these points, Maynard Smith¹⁶ and Kimura and Ohta¹⁷ have suggested that the model of stepwise allele production is more appropriate. Using this mutational scheme but assuming an infinite number of possible states, we have shown that the effective number of neutral alleles maintained in a finite population becomes, $n_e = \sqrt{(1 + 8N_e v)}$, where N_e is the effective population size and v is the mutation rate per gamete¹⁸. When $N_e v$ is large this formula gives a smaller effective allele number than that predicted by Kimura and Crow's model. Furthermore, the frequency distribution of multiple alleles is influenced significantly. We have performed extensive Monte Carlo experiments and have shown that the ratio of the effective to the actual number of alleles (n_e/n_a) is larger in the step allele model, particularly when n_a is large (T. O. and M. Kimura, unpublished). Then the agreement between the observation and Kimura and Crow's model shows that there exist more rare alleles than expected from the strict neutral hypothesis. From this, I conclude that rare alleles are on the

Table 1 Relationship between Substitution Rate in Small Populations and Equilibrium Frequency in Large Populations of Slightly Deleterious Mutations

Homozygous disadvantage	Equilibrium frequency in large populations	Rate of substitution in proportion of the completely neutral case $N_e = 10^2$	$N_e = 10^3$
Recessive deleterious			
10^{-2}	0.032	0.43	0.8×10^{-7}
10^{-3}	0.1	0.94	0.43
10^{-4}	0.32	0.99	0.94
Semidominant deleterious			
10^{-2}	0.002	0.31	0.5×10^{-7}
10^{-3}	0.02	0.90	0.31
10^{-4}	0.2	0.99	0.90

The mutation rate is assumed to be 10^{-5} .

average selected against. The alleles, which can increase their frequency up to several per cent in very large populations like *Drosophila* species, can replace the previous allele in small populations by random drift.

O'Brien *et al.*¹⁹ reported that the "null" alleles at the α -glycerophosphate dehydrogenase-1 locus of *Drosophila melanogaster* are deleterious. As their null alleles were induced by EMS, they may be different from those observed in low frequencies in natural populations. It is likely, however, that the null alleles are generally deleterious. They can replace the previous allele in a bottleneck population. Vitamin requirement is in fact considered to be the result of such procedures. King and Jukes² noticed that lack of vitamin C is always associated with the kind of food and they consider that, when selective constraints are reduced, the genes for vitamin C have deteriorated.

As an example, suppose that the homozygous disadvantage of the recessive deleterious mutant is 10^{-3} . If the mutation rate is 10^{-5} , the equilibrium mutant frequency is 10% in large populations. By using Kimura's²⁰ formula for the fixation probability of mutant genes, the rate of substitution of this slightly deleterious mutant becomes 0.43×10^{-5} per generation if the effective population size is 10^3 . This corresponds to 43% of the substitution rate of the strictly neutral case. Table 1 illustrates such examples in terms of the proportion of the substitution rate of the completely neutral case.

I have previously suggested the possibility of slightly deleterious mutant substitutions in cistrons in view of the apparently contradictory observation of generation-dependent DNA evolution against year-dependent cistron evolution⁴. Together with the supporting observations presented here, I suggest that very slight genetic deterioration might play an important role in molecular evolution. Here, organization at a much lower level in each molecule or in several interacting molecules could provide a clue to understanding the evolutionary change at the molecular level, rather than cohesive interaction of the pool often postulated by orthodox neo-Darwinism²¹. It seems from the report of Mukai *et al.*²² that there is no evidence that alleles at different loci constitute such interaction systems or "super genes". I thank Dr M. Kimura for discussions.

TOMOKO OHTA

National Institute of Genetics,
Mishima, Shizuoka-ken, 411

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¹ Kimura, M., *Nature*, **217**, 624 (1968).

² King, J. L., and Jukes, T. H., *Science*, **164**, 788 (1969).

³ Ohta, T., and Kimura, M., *J. molec. Evol.*, **1**, 18 (1971).

⁴ Ohta, T., *J. molec. Evol.*, **1**, 150 (1972).

⁵ Ohta, T., *J. molec. Evol.*, **1**, 305 (1972).

⁶ Fitch, W. M., and Margoliash, E., *Brookhaven Symp. Biol.*, **21**, 217 (1968).

⁷ Fitch, W. M., and Markowitz, E., *Biochem. Genet.*, **4**, 579 (1970).

⁸ Dayhoff, M. O., and McLaughlin, P. J., in *Atlas of Protein Sequence and Structure 1972* (edit. by Dayhoff, M. O.), 111 (National Biomedical Research Foundation, Silver Spring, 1972).

- ⁹ Jukes, T. H., *Biochem. biophys. Res. Commun.*, **24**, 744 (1966).
¹⁰ Barnard, E. A., Cohen, M. S., Gold, M. H., and Kim, Jae-Kyoung, *Nature*, **240**, 395 (1972).
¹¹ Ewens, W. J., *Theor. Pop. Biol.*, **3**, 87 (1972).
¹² Johnson, G. B., *Nature new Biol.*, **237**, 170 (1972).
¹³ Kimura, M., and Crow, J. F., *Genetics*, **49**, 725 (1964).
¹⁴ Yamazaki, T., and Maruyama, T., *Nature new Biol.* (in the press).
¹⁵ Bulmer, M. G., *Nature*, **234**, 410 (1971).
¹⁶ Maynard Smith, J., *Nature new Biol.*, **237**, 31 (1972).
¹⁷ Kimura, M., and Ohta, T., *Genet. Suppl.*, **73**, 19 (1973).
¹⁸ Ohta, T., and Kimura, M., *Genetical Res.* (in the press).
¹⁹ O'Brien, S. J., Wallace, B., and MacIntyre, R. J., *Am. Nat.*, **106**, 767 (1972).
²⁰ Kimura, M., *Genetics*, **47**, 713 (1962).
²¹ Mayr, E., *Animal Species and Evolution* (The Belknap Press of Harvard University Press, Cambridge, 1963).
²² Mukai, T., Mettler, L. E., and Chigusa, S. I., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1065 (1971).

Antitumour Activity of Iodine in Acidic Medium with Calcium

THE basal metabolism is increased in many patients with lymphatic and myelogenous leukaemia. Elevated levels of plasma iodine have been found in various leukaemic cases, possibly indicating involvement of the thyroid gland¹. It is thought that at lower pH values there is more free iodine, and so greater penetrability into cells and greater iodine activity². Iodine fixation by human leukocytes requires energy³. Iodine alters the redox state of the cytoplasm and mitochondria, because the couple $2\text{I}^- \rightarrow \text{I}_2 + 2\text{e}^-$ has a rather high redox potential: +0.535 V; iodine seems to be one of the oxidizing agents which can be used for ATP formation⁴. Flavin is the most likely oxidizing agent for a couple with such a high redox potential⁵.

The importance of calcium ions in controlling the permeability of cells⁶ and in regulating cellular activity⁷ has been established. The penetration of isolated ascites tumour cells by calcium ions depends on the metabolic state of the cells⁸; calcium accumulation, like iodine accumulation, requires energy and alters the redox state of the respiratory chain components.

It has been suggested that iodine in acidic medium with calcium has antitumour activity (L. Heinonen, personal communication). We therefore studied the effect of these substances against myelocytic chloroleukaemia⁹. This leukaemia was induced in 2 to 3-week-old Sprague-Dawley and Wistar rats by intraperitoneal or subcutaneous inoculation. Chloroleukaemic cells were suspended in physiological saline and 0.1 ml of the cell suspension, containing 10,000–20,000 cells, was injected into the rats. Survival time, histological examination and lactic acid dehydrogenase (LDH) activity were used to evaluate antitumour activity.

Treatment was started 24 h after the intraperitoneal inoculation by giving each rat about 500 µg of iodine daily as a 5% iodine-ethanol solution in cultured milk. Fourteen days after the inoculation the treatment with iodine was continued by subcutaneous injection. Treatment of the subcutaneously induced local tumours was by subcutaneous injection of iodine, starting about two weeks after inoculation, when the tumour was clearly visible and was usually about 2 cm × 3 cm, sometimes larger. The subcutaneous injections consisted of 500 µg of iodine in ethanol solution mixed with 1 ml of liver extract (Pernaemon forte, N.V., Organon, Oss, Netherlands). Immediately afterwards 1 ml of calcium gluconolactobionate solution (about 9 mg Ca²⁺) (Calcium Sandoz, Sandoz AG, Basel, Switzerland) was injected intramuscularly. The injections were made once (intraperitoneally inoculated rats) or twice (subcutaneously inoculated rats) daily. Eleven days after

inoculation the intraperitoneally inoculated rats were injected with 25 mg of thiamine chloride (Neuramin forte, Orion Ltd., Espoo, Finland).

The rats' food consisted of vegetables, including cabbage and carrots, and fresh liver, with 3 mg riboflavin (Orion Ltd., Espoo, Finland) per rat per day. The control rats were given all the nutrients, liver extract, calcium and vitamins but not iodine.

Tissue samples were taken at autopsy not only from the visible tumours but also as a routine procedure from liver, spleen, kidney, lung, heart and bone marrow. After fixation in formalin the tissue samples were cut into sections 6 µm thick and stained with haematoxylin-eosin. The smear samples of femoral bone marrow were stained by the May-Grünwald-Giemsa method. The preparations were examined under a light microscope. LDH activity was estimated by measuring the extent of oxidation of NADH (at 340 nm).

Table 1 shows the effect of treatment on the survival time of the rats bearing a tumour inoculated intraperitoneally or subcutaneously. The index $T/C \times 100$ [(mean survival time of treated rats)/(mean survival time of untreated control rats) × 100] was calculated to be >415 for the intraperitoneally inoculated rats. This is three to four times higher than the ratio reported by other workers^{10–12}. Our result might have been even better if the iodine administration had been by injection from the start of the experiment, as we found that the rats did not drink all of their iodine-containing cultured milk. The index was not calculated for the subcutaneously inoculated rats because the tumours had disappeared before the rats were killed (Tables 1 and 2). The average survival time

Table 1 Influence of Iodine Treatment on Survival Time

Intraperitoneally inoculated rats					
Rat	Survival time T (d after inoculation)	$T/C \times 100$	Cause of death	No. of treatments	Dose of iodine (µg d ⁻¹)
Treated rats					
a	> 61	> 555	Killed	25	500
b	28	255	Died	17	500
c	17	155	Died	3	500
d	> 61	> 555	Killed	25	500
e	> 61	> 555	Killed	26	500
Untreated control rats					
f	12	—	Died	—	—
g	12	—	Died	—	—
k	9	—	Died	—	—
Subcutaneously inoculated rats (local tumour)					
Rat	Survival time (d after inoculation)	Cause of death	No. of treatments	Dose of iodine (µg d ⁻¹)	Disappearance of the local tumour (d)
Treated rats					
II†	> 198	Killed	68	625	14
q‡	> 33	Killed	35	1,000–4,500	†
r	> 33	Killed	24	1,000	3
s	> 33	Killed	13	1,000	2
v	> 33	Killed	12	1,000	3
Untreated control rats					
p	> 33	Killed	—	—	—
XI	24	Died	—	—	—
j	35	Died	—	—	—
ö	34	Died	—	—	—
y	35	Died	—	—	—
z	41	Died	—	—	—

* C, Mean survival time of untreated control rats (f–k) = 11 d and of untreated control rats (XI–z) = 34 d.

† Rat II received iodine in cultured milk during the whole experimental period.

‡ Rat q received a more concentrated iodine-liver extract daily (1,000 to 4,500 µg of iodine ml⁻¹). The tumour underwent alteration to fatty necrosis.

of the control rats inoculated subcutaneously was 34 d from inoculation, somewhat longer than the survival time reported by Rytömaa and Kiviniemi¹³. All the five rats with local tumours became healthy after iodine treatment, and eight out of the total number of ten treated rats recovered, whereas eight out of the nine untreated control rats died.

Table 2 Effect of Treatment on the Size of Subcutaneously Induced Tumours

Rat	Diameter of tumour at beginning of treatment (cm)	Fate of tumour
Treated rats		
II		Complete disappearance in 14 d
q	2.8*	Transformation to fatty necrosis
r	2.0†	90% reduction in 2 d, complete disappearance in 3 d
s	1.5	Complete disappearance in 2 d
v	2.5	Complete disappearance in 3 d
Untreated control rats		
p	2.8	Growth ceased after 5 d
j	2.8	Growth continued
XI	1.5	Growth continued

* This tumour was treated by pouring over the tumour a large amount of iodine in liver extract, followed by the calcium solution.

† Two subcutaneous inoculations resulted in two tumours that grew together over the neck for a length of 4 cm.

The histological findings are presented in Table 3. Leukaemic infiltrations were found in only two of the ten treated rats. In the untreated group of eight rats there was tumour tissue in seven animals. Recovery was 100% from subcutaneously induced tumours and 60% from intraperitoneally induced tumours. One local large tumour (rat q) was treated by pouring over the tumour a large amount of iodine in liver extract, followed by the calcium solution. In its histological examination only fatty necrosis was seen. During the recovery of the treated rats the number of erythrocytes, the haemoglobin value, and the packed cell volume attained normal levels.

Elevated LDH activity has been found in a variety of tissue tumours and in acute and chronic myelocytic

Table 3 Histological Findings in Different Organs at Autopsy of Treated and Untreated Rats Inoculated Intraperitoneally or Subcutaneously with Leukaemic Cell Suspension (Myelocytic Chloroleukaemia)

Leukaemic infiltrations	Treated rats		Total No. of rats
	Intraperitoneal inoculation (rats: a, b, c, d, e) No. of rats	Subcutaneous inoculation (rats: II, q, r, s, v) No. of rats	
Yes	2	—	2
No	3	5	8
Total	5	5	10
Leukaemic infiltrations	Untreated control rats		Total No. of rats
	Intraperitoneal inoculation (rats: g, i, k) No. of rats	Subcutaneous inoculation (rats: p, XI, ö, y, z) No. of rats	
Yes	3	4	7
No	—	1	1
Total	3	5	8

leukaemia¹⁴. We measured the LDH activity in the pooled peripheral blood of treated rats (Table 4). In the rats with subcutaneous tumours the activity of LDH decreased during the treatment and was close to, though still above, the control value for normal healthy rats at the end of the treatment. The two leukaemic rats which did not recover are presumably the source of the elevated value in the pooled samples. The effectiveness of iodine in lowering LDH activity may be due to the fact that iodine rapidly oxidises the SH groups of LDH¹⁵, inhibiting the enzyme activity.

Table 4 LDH Activity in Peripheral Blood During the Treatment

Form of leukaemia	Stage of treatment			Duration of experiment (d)
	Beginning (before treatment)	Middle	End	
Intraperitoneally inoculated	8,000 (n=0)	6,490 (n=3)	4,630 (n=11)	5
Subcutaneous tumour	2,140 (n=0)	460 (n=10)	860 (n=16)	16
Normal healthy rats	720 (n=0)		650 (n=0)	16

n, No. of treatments.

LDH activity is given in Wroblensky units per ml.

Four rats on average were used for each determination.

The average body weight of the intraperitoneally inoculated and treated rats increased gradually from 30 g to 195 g in the month following inoculation, while the untreated rats showed only a small increase in weight (22 g) during their lifetime. After the disappearance of the local tumours from the subcutaneously implanted rats, their weight increased normally.

From these results it can be concluded that iodine in acidic medium with calcium has a remarkable activity against myelocytic chloroleukaemia. Iodine may act on the electron transport chain: mitochondria in tumour cells differ morphologically and functionally from normal cells. Most suggestions for the mechanism of action of anti-tumour agents have involved inhibition of DNA and protein synthesis, whereas we suggest that iodine acts by changing the redox state inside the cell.

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HILKKA VOLTTI
R. S. PIHA

Department of Biochemistry,
University of Oulu,
Oulu

M. ALAVAikko

Division of Pathology,
Central Hospital,
Kokkola

I. P. PALVA

Department of Medicine,
University of Oulu,
Oulu

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- ¹ Cantarow, A., and Trumper, M., in *Clinical Biochemistry*, sixth ed. (Saunders, Philadelphia and London, 1962).
- ² Webb, J. L., in *Enzyme and Metabolic Inhibitors*, 2 (Academic, New York and London, 1966).
- ³ Stolz, V., *Biochim. biophys. Acta*, **264**, 285 (1972).
- ⁴ Bäuerlein, E., Klingenfuss, M., and Wieland, T., *Eur. J. Biochem.*, **24**, 308 (1971).
- ⁵ Thompson, R. H. S., and Wootton, I. D. P., in *Biochemical Disorders in Human Disease*, third ed. (Churchill, London, 1970).
- ⁶ Rasmussen, H., *Science, N.Y.*, **170**, 404 (1970).
- ⁷ Bygrave, F. L., *Nature*, **214**, 667 (1967).
- ⁸ Cittadini, A., Scarpa, A., and Chance, B., *FEBS Lett.*, **18**, 98 (1971).
- ⁹ Shay, H., Gruenstein, M., and Harris, C., *Acta haemat.*, **14**, 337 (1955).
- ¹⁰ Hodnett, E. M., Moore, C. H., and French, F. A., *J. med. Chem.*, **14**, 1121 (1971).
- ¹¹ Hodnett, E. M., and Tai, J., *J. med. Chem.*, **14**, 1115 (1971).
- ¹² Kupchan, S. M., Eakin, M. A., and Thomas, A. M., *J. med. Chem.*, **14**, 1147 (1971).
- ¹³ Rytömaa, T., and Kiviniemi, K., *Nature*, **222**, 995 (1969).
- ¹⁴ Hess, B., in *Enzyme im Blutplasma*, first ed. (G. Thieme Verlag, Stuttgart, 1966).
- ¹⁵ Nygaard, A. P., *Acta chem. scand.*, **10**, 397 (1956).

Vitamin D-induced Synthesis of mRNA for Calcium-binding Protein

THE discovery that vitamin D must first be converted into the metabolites 25-hydroxycholecalciferol¹ (25 OH-D₃) and 1,25-dihydroxycholecalciferol² (1,25(OH)₂-D₃) before calcium absorption across the small intestine is enhanced has radically changed existing ideas as to the mode of action of vitamin D. Indeed, it is now well established that the most active metabolite of vitamin D for calcium absorption, 1,25(OH)₂-D₃, is produced solely by the kidney³ and that its production and secretion are under very fine endocrine control. This finding, together with the observation that 1,25(OH)₂-D₃ is specifically located in the nucleus of the target cell⁴ and the apparent involvement of RNA and protein synthesis⁵⁻⁷ in the intestinal action of vitamin D, provide the basis of the proposal that 1,25(OH)₂-D₃ can be classed as a steroid hormone.

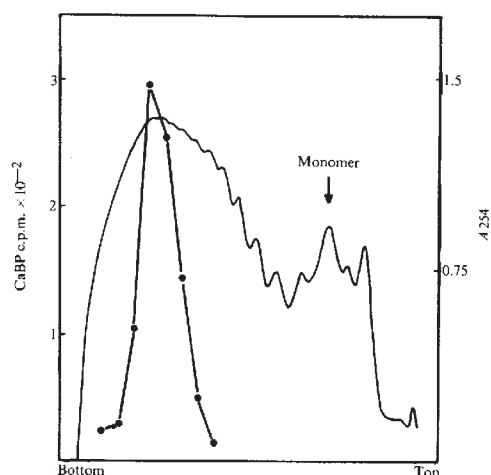


Fig. 1 Size of CaBP-synthesizing polysomes. 1 mg (20 A_{260} units) of polysomes from intestinal cells of vitamin D₃-dosed chicks was layered on a 15 to 45% sucrose gradient in 25 mM NaCl, 25 mM Tris-HCl, pH 7.5, 5 mM MgCl₂ and centrifuged in the SW 36 rotor for 100 min at 2° C and 35,000 r.p.m. Fractions were collected as indicated and RNA extracted and translated in the lysate as described in Table 2. ●, CaBP synthesizing polysomes; —, polysome profile.

The role of 1,25(OH)₂-D₃ as a steroid hormone implies that it acts at the level of gene transcription. We have tested this hypothesis by investigating the synthesis of calcium binding protein (CaBP), formed in response to vitamin D⁸. More specifically, we have sought to determine whether CaBP is synthesized *de novo* or formed from an inactive precursor as proposed by Drescher and DeLuca⁹ and whether the action of 1,25(OH)₂-D₃ on the nucleus promotes the formation of a specific mRNA for CaBP.

Table 1 Effect of Vitamin D on the Synthesis of CaBP by Isolated Polysomes

Source of polysomes	TCA insoluble d.p.m. in released protein	Radioactivity in immunoprecipitate (d.p.m.)
Rat liver	56,000	330
Chick intestinal mucosa (–D)	51,200	270
Chick intestinal mucosa (+D)*	64,000	4,475

* Rachitic chicks received 5 µg vitamin D₃ 72 h before being killed.

Polysomes were isolated as described¹⁰. *In vitro* polypeptide synthesis was conducted at 37° C in a medium containing 1 mM ATP, 0.4 mM GTP, 10 mM creatine phosphate, 40 µg ml⁻¹ creatine kinase, 50 µM of each amino acid except leucine, 0.5 µCi ml⁻¹ ³H-leucine, 0.2 to 0.5 mg ml⁻¹ polysomal RNA, 0.2 ml ml⁻¹ gel-filtered rat liver cell sap, 1 mM dithiothreitol and 20 mM Tris-Cl, pH 7.5. Optimal ionic concentrations were 4 mM MgCl₂ and 100 mM KCl. After 30 min incubation, ribosomes were removed by centrifugation at 150,000g (maximum) for 4 h to produce a supernatant containing released protein. Trichloroacetic acid (TCA) precipitable radioactivity was measured as described by Mans and Novelli¹⁴. Immunoprecipitates were washed twice and dissolved in 0.2 ml NCS tissue stabiliser for scintillation counting.

Table 2 Synthesis of Immunoprecipitable Protein in a Rabbit Reticulocyte Lysate Programmed with Intestinal RNA

RNA added	Total radioactivity incorporated (c.p.m. per 10 µl)	¹⁴ C precipitated by anti-CaBP (c.p.m.)
None	14,200	149
150 µg (–D)	13,700	155
108 µg (+D)*	14,300	361
216 µg (+D)*	14,000	551

* Rachitic chicks received 5 µg vitamin D₃ orally 72 h before being killed.

Incubation conditions for the lysate were as described¹⁵ except that ATP and GTP were omitted and MgCl₂ and creatine phosphate were present at 0.5 mM and 10 mM respectively; the concentration of non-radioactive leucine was 50 µM and ¹⁴C-leucine was 2.5 µCi ml⁻¹. Incubations were carried out at 30° C for 60 min in a volume of 500 µl and contained the amounts of intestinal RNA shown above. After incubation, a 10 µl sample was taken to measure TCA insoluble radioactivity¹⁶; the remainder was made 1% with Triton-X100 and deoxycholate and 10 mM with leucine. Non-radioactive CaBP (10 µg) and excess antiserum were then added to the mixture and, after 30 min at room temperature, the immunoprecipitate was collected by centrifugation through M sucrose¹¹ and dissolved in NCS tissue solubiliser.

The first question was answered by investigating the ability of polysomes isolated from rachitic and vitamin D₃-dosed chicks to synthesize CaBP in an *in vitro*, cell-free system. Chick intestinal polysomes were isolated from a post-mitochondrial supernatant by sedimentation through a 0.5 M/2.0 M discontinuous sucrose gradient as previously described¹⁰ and incubated as shown in Table 1. After the

incubation, the ribosomes were removed by centrifugation and the incorporation of ^3H -leucine into completed polypeptide chains and into CaBP was determined. The specificity of the precipitin reaction used to quantify CaBP synthesis has already been reported¹⁰. It is clear from Table 1 that tritium is incorporated into CaBP by polysomes isolated from vitamin D₃-dosed chicks. On the other hand, polysomes both from rachitic chick intestinal cells and from rat liver cells resulted in the same low level of radioactivity in the immunoprecipitate. We interpret these results as *de novo* synthesis of CaBP.

We wished to test directly the production of a specific mRNA as a result of vitamin D administration. RNA was prepared from intestinal polysomes (for method see ref. 11) and incubated in a rabbit reticulocyte lysate¹⁵. Table 2 shows that RNA preparations from intestinal polysomes of vitamin D-dosed chicks directed the *in vitro* synthesis of a product which is precipitable by our specific antiserum. RNA from rachitic chick intestinal polysomes was devoid of this activity.

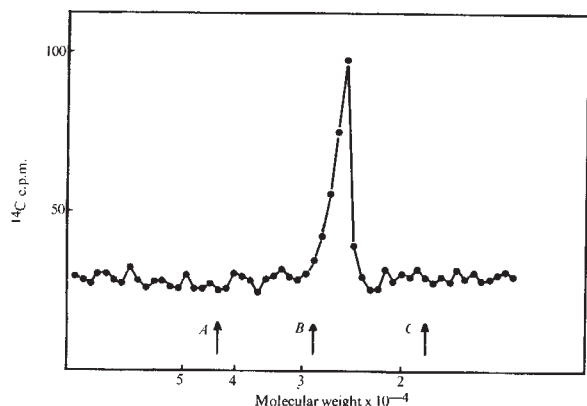


Fig. 2 Sodium dodecylsulphate-acrylamide gel electrophoresis of anti-CaBP precipitable radioactivity. RNA from the peak fraction of Fig. 1 was translated in the lysate and the immunoprecipitable radioactivity recovered as described in Table 2. The immunoprecipitate was dissolved by heating to 100°C for 4 min in a sodium dodecylsulphate-Tris-dithiothreitol solution¹⁷ and then electrophoresed on sodium dodecylsulphate-acrylamide gels. Gels were prepared as described by Laemmli¹⁸ except that the concentration of bis-acrylamide was doubled. Electrophoresis was at 5 mA per gel for 100 min. After electrophoresis, 1 mm slices were cut and incubated at 50°C for 2.5 h with 500 μl NCS tissue solubiliser and 30 μl H₂O. After cooling, they were counted in a 10 ml toluene scintillator. Also shown in the figure are the electrophoretic mobilities of, A, ovalbumin (molecular weight 43,000); B, carbonic anhydrase (molecular weight 29,000); C, myoglobin (molecular weight 17,300) on parallel gels.

It was also of interest to determine the size of the CaBP-synthesizing polysomes as well as the molecular weight of the protein produced by the lysate. Thus, intestinal polysomes were fractionated on a sucrose gradient and RNA prepared, as described above, from the fractions. This RNA was translated in the lysate and immunoprecipitable material recovered. Figure 1 displays the polysome profile and the distribution of the CaBP-synthesizing polysomes. It can be seen that CaBP is synthesized on polysomes containing between eleven and fourteen ribosomes. This finding was rather surprising as ovalbumin (387 amino acids)¹² is synthesized on polysomes of the same size¹³. Indeed, we expected to find that CaBP (206 amino acids)⁸ was synthesized on polysomes containing six to eight ribosomes. This anomaly may be explained by postulating that either elongation is rate limiting or that a larger protein is produced and then cleaved after or during synthesis to yield an active CaBP. These possibilities are at present under investigation.

Finally, an experiment was performed to characterise the protein synthesized under the direction of intestinal RNA.

When this RNA was added to the lysate and the resulting antibody precipitate analysed by electrophoresis on SDS-acrylamide gels, a single peak of radioactivity was observed (Fig. 2). Moreover, the mobility of this peak corresponded to a molecular size of 26,000 daltons and was similar to that of authentic CaBP electrophoresed on parallel gels. The absence of a peak of radioactivity with a large molecular weight argues against the precursor molecule discussed above.

We have demonstrated the *de novo* synthesis of a protein having similar molecular and antigenic properties as the intestinal CaBP. The ability to synthesize this protein resides in both polysomes and RNA of intestinal cells from vitamin D₃-dosed chicks; polysomes or RNA from rachitic chicks do not possess this ability. Thus, this study supports the concept that the active metabolite of vitamin D acts in an analogous fashion to the classical steroid hormones.

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J. S. EMTAGE*
D. E. M. LAWSON
E. KODICEK†

Dunn Nutritional Laboratory,
University of Cambridge and Medical Research Council,
Milton Road, Cambridge.

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*Present address: G. D. Searle and Co. Ltd, High Wycombe, Buckinghamshire.

†Present address: Strangeways Research Laboratory, Cambridge.

- 1 Blunt, J. W., DeLuca, H. F., and Schnoes, H. D., *Biochemistry N.Y.*, **7**, 3317 (1968).
- 2 Lawson, D. E. M., Fraser, D. R., Kodicek, E., Morris, H. R., and Williams, D. H., *Nature*, **230**, 228 (1971).
- 3 Fraser, D. R., and Kodicek, E., *Nature*, **228**, 764 (1970).
- 4 Lawson, D. E. M., Wilson, P. W., and Kodicek, E., *Nature*, **222**, 171 (1969).
- 5 Zull, J. E., Czarnowska-Misztal, E., and DeLuca, H. F., *Science*, **N.Y.**, **149**, 182 (1965).
- 6 Norman, A. W., *Science*, **N.Y.**, **149**, 184 (1965).
- 6a Lawson, D. E. M., Wilson, P. W., Barker, D. C., and Kodicek, E., *Biochem. J.*, **115**, 263 (1969).
- 7 Corradino, R. A., *Nature*, **243**, 41 (1973).
- 8 Wasserman, R. H., Corradino, R. A., and Taylor, A. N., *J. biol. Chem.*, **243**, 3978 (1968).
- 9 Drescher, D., and DeLuca, H. F., *Biochemistry, N.Y.*, **10**, 2308 (1971).
- 10 Lawson, D. E. M., and Emtage, J. S., in *The Metabolism and Function of Vitamin D* (Biochemical Society, London, in the press).
- 11 Palmiter, R. D., *J. biol. Chem.*, **248**, 2095 (1973).
- 12 Neuberg, A., and Marshal, R. D., in *Glycoproteins* (edit. by Gottschalk, A.), **5**, 299 (BBA Library, Elsevier, New York, 1966).
- 13 Palmiter, R. D., Palacios, R., and Schimke, R. T., *J. biol. Chem.*, **247**, 3296 (1972).
- 14 Mans, R. J., and Novelli, G. D., *Archs Biochem. Biophys.*, **94**, 48 (1961).
- 15 Darnbrough, C., Legon, S., Hunt, T., and Jackson, R. J., *J. molec. Biol.*, **76**, 379 (1973).
- 16 Jackson, R. J., and Hunter, A. R., *Nature*, **227**, 672 (1970).
- 17 Palmiter, R. D., Oka, T., and Schimke, R. T., *J. biol. Chem.*, **246**, 724 (1971).
- 18 Laemmli, U. K., *Nature*, **227**, 680 (1970).

Abrogation of Lymphocyte Trapping by Ascitic Tumours

SEQUESTRATION of lymphocytes within antigen-stimulated lymphoid organs is observed regularly following injection of immunogens and adjuvants¹⁻⁵. This lymphoid "trapping" is one of the earliest events in immunization and may be necessary for the expression of an effective immune response. The

mechanism responsible for controlling the operation of the "trap" is incompletely understood but must relate to the way in which lymphocytes normally recirculate.

We have observed defective lymphocyte trapping in animals bearing ascitic tumours. This defect could be transferred to normal animals by injections of cell-free ascitic fluid from animals bearing tumours. The abrogation of trapping applied not only to the tumour but to all immunogens and adjuvants tested. These observations may have relevance to the immunosuppressed state widely recorded in animals and humans bearing advanced tumours⁶⁻⁸ and to the ineffective immune response towards the tumour itself.

The method used to demonstrate lymphocyte trapping has been described in detail elsewhere¹⁻³. The extent to which lymphocytes are sequestered within antigen-stimulated lymphoid organs is measured by determining localization of ⁵¹Cr-labelled syngeneic lymph node cells 24 h after intravenous injection into animals bearing tumours or challenged with antigen. The extent of trapping is reflected by the degree to which the lymphocytes are retained in stimulated lymphoid organs in excess of the localization in untreated controls.

Groups of Balb/c mice received either 10⁷ S37 or 5 × 10⁷ Meth/A sarcoma cells intraperitoneally or were uninjected. At four daily intervals thereafter radio-labelled syngeneic lymph node cells were injected intravenously into representatives of each group and the localization of label determined 24 h later. Animals bearing intraperitoneal tumours had considerably greater localization of labelled cells in their spleens on days 1 and 2 than controls (Table 1). The distribution of labelled cells became identical to that in the controls by day 2 in the Meth/A bearing animals and by day 3 in the S37 bearing animals. (The difference of 1 d between the groups may be related to the higher dose of Meth/A tumour cells used.) Therefore in spite of the continued presence of antigenic tumour, trapping in the spleen was evanescent.

When Balb/c mice received subcutaneous implants of either tumour, augmented localization of radio-labelled cells

occurred in the draining lymph nodes. In contrast to animals bearing ascitic tumours, trapping in this case did not decay but was continuously present for as long as 40 d following implantation. Furthermore, these animals were still able to "trap" when challenged with antigen intravenously (spleen) or subcutaneously (contralateral nodes).

The failure of splenic trapping in mice bearing ascitic tumours for more than 48 h might be explained in several ways. The rapid multiplication of tumour cells in the peritoneal cavity could induce immunological non-reactivity to tumour antigen; trapping is known not to occur in tolerant mice⁹. In this case the loss of trapping would be immunologically specific. Alternatively the presence of intraperitoneal tumour might exert some general effect on the kinetics of lymphocyte circulation. To differentiate between these possibilities animals bearing ascitic tumours were challenged with antigens known to be highly effective in eliciting trapping¹⁻³ and their responses compared with those of normal controls (Table 2). The intravenous injection of sheep erythrocytes (sheep RBC), keyhole limpet haemocyanin (KLH) or Salmonella "H" antigen caused marked sequestration of radio-labelled lymphocytes in the spleens of normal animals. Animals which bore ascitic tumours (4 d after inoculation) could not produce this response. (Although not shown in Table 2, this ability to cause failure of trapping was demonstrated with Meth/A tumour as well.) A similar discrepancy was found after subcutaneous challenge with the same battery of antigens: animals bearing ascitic tumours did not show trapping in the antigen-stimulated lymph nodes.

The latent interval between peritoneal tumour administration and trapping abrogation was indirectly related to the dose of injected tumour cells. Animals receiving an initial dose of 10⁶ tumour cells continued to trap for 5 d, whereas at a high dose of tumour (10⁷ cells) trapping ceased by day 3. Antigen challenge in these animals showed a non-specific failure of trapping by day 4 in the animals given 10⁷ tumour cells. In animals given 10⁶ tumour cells the subcutaneous challenge by antigen failed to initiate trapping by day 7 while intravenous trapping failed by day 9.

Table 1 The Effect of Injection of Intraperitoneal Tumour on Lymphocyte Trapping

	Day 1		Day 2		Day 3		Day 4	
S37 sarcoma	Control	IP tumour	Control	IP tumour	Control	IP tumour	Control	IP tumour
% Localization of labelled cells in the spleen	17.2 ± 0.9	28.1 ± 1.3	16.5 ± 0.5	22.0 ± 1.5	12.6 ± 0.1	11.8 ± 0.3	12.6 ± 0.5	8.6 ± 0.6
% Trapping		63		33				
Meth/A sarcoma								
% Localization in the spleen	17.8 ± 1.0	23.8 ± 1.6	18.5 ± 2.5	20.0 ± 2.4	15.9 ± 0.8	16.2 ± 1.2		
% Trapping*		33		12		0		

Groups of mice injected with 10⁷ S37 or 5 × 10⁷ Meth/A tumour cells on day 0. On days 1-4 control and tumour-bearing animals were injected with ⁵¹C-labelled lymphocytes and the percentage of splenic localization of these cells was determined.

* Trapping of less than 20% is not considered significant.

Table 2 The Failure of Lymphocyte Trapping in Ascitic Tumour-bearing Mice (4 d)

	No antigen		Sheep RBC		Keyhole limpet haemocyanin		Sal. H suspension	
S37 tumour	Control	Tumour bearers	Control	Tumour bearers	Control	Tumour bearers	Control	Tumour bearers
% Localization in the spleen	15.3 ± 0.7	18.6 ± 0.6	27.7 ± 1.6	19.8 ± 0.3	28.2 ± 0.4	16.5 ± 0.6	25.4 ± 0.7	16.8 ± 0.4
% Trapping			81%	0	84%	0	66%	0

Mice bearing ascitic tumour (10⁷ given intraperitoneally on day 0), and controls, were challenged with SRBC, KLH or Sal. H given intravenously 1 h before labelled lymph node cells.

Table 3 The Failure of Lymphocyte Trapping in Supernatant Treated Animals

% Localization of labelled cells	Antigenic challenge		
	No antigen	Subcutaneous sheep RBC	Intravenous sheep RBC
Left lymph nodes	2.6±0.1	2.1±0.1	
Right lymph nodes	2.7±0.2	2.1±0.1	9.1±0.9
Mesenteric lymph nodes	7.1±1.1	4.9±0.3	
Spleen	18.0±1.0	15.1±0.3	19.7±0.9
Liver	28.4±1.8	23.2±0.9	17.1±0.6

Three groups of Balb/c mice were treated with 0.5 ml ($\times 3$) of supernatant raised by injection of 10^7 Meth/A tumour cells in syngeneic hosts. Animals were then challenged with antigen, subcutaneously, on day 3 and intravenously on day 4: one group was left unchallenged. ^{51}Cr -labelled lymph node cells were injected on day 4 and their percentage localization determined on day 5.

Because failure of lymphocyte trapping occurred only in animals bearing intraperitoneal tumour and affected responses not only locally but also in peripheral sites, we considered that the effect might be mediated by some tumour cell product or host factor liberated into the ascitic fluid. To test this, ascitic fluid from tumour-bearing mice was removed and centrifuged to remove cells in suspension. The cell-free supernatant was injected in varying dosages either intravenously or subcutaneously into normal Balb/c mice which were then challenged with antigen or adjuvants known to elicit trapping in untreated controls. Animals which received ascitic fluid supernatant failed to show trapping either in the spleen after intravenous antigen challenge or in the draining lymph nodes after subcutaneous antigen challenge (Table 3). Abrogation of subcutaneous trapping was observed after as little as three daily 0.1 ml intravenous doses of supernatant or a single intravenous injection of 0.3 ml. The consistent abrogation of splenic trapping requires a somewhat higher dose of supernatant, 1 ml $\times 1$ or 0.5 ml $\times 3$. This effect lasted between 24 and 48 h after three pulses of supernatant. Animals treated with supernatant also failed to demonstrate trapping in the regional lymph nodes when challenged with subcutaneous inoculation of the same tumour.

Preliminary observations suggest that the activity present in cell-free ascitic fluid supernatant is preserved after heating or freezing. Samples heated to 56° C for 30 min, or stored at -20° C for up to 3 months, or spun at 100,000g for 3 h, were still active in ablating trapping. The activity is also known to cross allogeneic barriers, that is, supernatant raised in Balb/c mice is effective in abrogating trapping in C3H mice.

If the ability to recruit lymphocytes is important for initiation of immune responses then interference with this mechanism should produce an immunosuppressive effect. The survival of DBA skin grafts on Balb/c mice was prolonged when graft recipients were treated with ascitic supernatant. The median survival time of such grafts in mice treated with 0.3 ml amounts of supernatant for 3 d was 16 d compared with 12 d in untreated controls (unpublished).

These results establish that animals bearing ascitic sarcomas develop impaired ability to recruit lymphocytes after antigen challenge. This defect is immunologically non-specific and can be adoptively transferred to normal animals by a cell-free ascitic fluid supernatant. Such a defect would be expected to result in impaired immune responsiveness, a prediction tentatively supported by the preliminary results of allogeneic skin grafting.

The observation that ascitic fluid from animals bearing tumours may have immunosuppressive properties is not new. Depression of the response to sheep erythrocytes by the administration of tumour ascitic fluid has been reported *in vivo* by Yamazaki *et al.*¹⁰ and *in vitro* by Chan and Sinclair¹¹. Apfel *et al.*¹² found that daily aspiration of ascitic fluid had a protective effect against tumours, and Groshman

and Nowotny¹³ reported that ascitic fluid antagonized the protective effect of endotoxin on tumour growth.

Whether the alteration in lymphocyte kinetics induced by tumour ascitic fluid is directly responsible for immunosuppression remains to be established. These observations offer, however, a possible explanation for the generalized depression of immune responsiveness widely recorded in animals and man with advanced cancer and provide a method for investigating this phenomenon. Moreover, these results suggest yet another mechanism whereby some tumours may escape or render ineffective the immune surveillance and destructive defence of the host. The extent to which these speculations apply to some or all tumours remains to be elucidated.

PHILIP FROST
EUGENE M. LANCE

*Division of Surgical Sciences,
Clinical Research Centre,
Watford Road,
Harrow, Middlesex HA1 3UJ*

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- 1 Frost, P., and Lance, E. M., *Immunology* (in the press).
- 2 Frost, P., and Lance, E. M., in *Ciba Symposium—Immunopotential* (edit. by Wolstenholme, G. C. W., and Knight, J.) (Churchill, London, in the press).
- 3 Zatz, M., and Lance, E. M., *J. exp. Med.*, **134**, 274 (1971).
- 4 Dresser, D. W., Taub, R. N., and Krantz, G. A. R., *Immunology*, **18**, 663 (1970).
- 5 Ford, W. L., *Immunology*, **15**, 609 (1968).
- 6 Southam, C. N., *Cancer Res.*, **28**, 1433 (1968).
- 7 Harris, J., and Bagai, R. C., *Med. Clin. N. Am.*, **56**, 501 (1972).
- 8 Bianco, G., Braun, B. L., Jones, E. E., and Rosenoer, V. M., *Proc. Soc. exp. Biol. Med.*, **136**, 507 (1971).
- 9 Zatz, M., and Lance, E. M., *Nature new Biol.*, **234**, 253 (1971).
- 10 Yamazaki, H., Nitta, K., and Umezawa, H., *Gann*, **64**, 83 (1973).
- 11 Chan, C. L., and Sinclair, N. R. St C., *J. natn. Cancer Inst.*, **48**, 1829 (1972).
- 12 Apfel, C. A., Arnason, B. G., Twinam, C. W., and Harris, C. A., *Br. J. Cancer*, **20**, 122 (1966).
- 13 Groshman, J., and Nowotny, A., *J. Immun.*, **109**, 1090 (1972).

New Histone Kinases in Nuclei of Rat Tissues

THE presence of acid-stable phosphomonoesters, principally as phosphoserine, in histones has been reported by several investigators¹⁻⁴, and histone kinases which phosphorylate serine in histones I and IV have been studied widely⁵⁻⁷. Many of the histone kinases studied are stimulated by cyclic AMP^{5,8}.

In examining nuclear extracts of Walker-256 carcinosarcoma for histone kinases we have found that such extracts supplemented with whole calf thymus histones and γ - ^{32}P -ATP yield a ratio of acid-labile to acid-stable protein phosphates from 2 to 3.5, dependent on pH. Further, we have found that histones I and IV are phosphorylated to produce acid-labile phosphates by at least two separate enzymes obtained from Walker-256 carcinosarcoma nuclear extracts and similar extracts of normal rat tissues. The new kinases are apparently independent of cyclic AMP.

The new histone kinases were assayed in an incubation volume of 0.2 ml, containing: glycine-NaOH buffer (pH 9.5), 10 μmol , or maleate buffer (pH 6.5), 10 μmol ; γ - ^{32}P -ATP, 0.03 μmol , containing about 1.5×10^6 c.p.m.; magnesium acetate, 4 μmol ; ethylene glycol bis-(β -aminoethyl ether)-N,N'-tetraacetic acid, 0.03 μmol ; and histone IV or histone I, 20 μg . The reactions were started by addition of enzyme, and incubations were carried out for 5 min at 30° C. The reactions were terminated by the addition of 88% phenol buffered with 0.01 M sodium phosphate to pH 8, and the acid-labile ^{32}P bound to protein was determined by the phenol extraction-acetone precipitation method of Boyer and Bieber⁹.

Nuclei from all tissues studied except brain and regenerated rat liver were prepared by the method of Chauveau *et al.*¹⁰ except that $MgCl_2$ was used in place of $CaCl_2$. Brain nuclei were prepared by the method of Lovtrup-Rein and McEwen¹¹, and regenerated rat liver nuclei were prepared by the method of Higgins and Anderson¹².

Crude extracts from the Walker-256 carcinosarcoma nuclei, when assayed for the formation of acid-labile histone phosphates, showed a bimodal pH profile with one peak of activity at pH 9.5 (the 'pH 9.5 kinase') and another peak of activity at pH 6.5 (the 'pH 6.5 kinase'). Nuclei from rat spleen and from regenerated rat liver had activities similar to those of Walker-256 carcinosarcoma (Fig. 1a), while nuclei obtained from rat liver and from rat brain had only the pH 6.5 activity (Fig. 1b). Kidney nuclei have an apparently unique enzyme with a pH optimum about 8.7 (Fig. 1b).

The new histone kinases are principally found in the acidic protein fraction prepared from nuclei by the method of Wang¹³ and have been further purified from this fraction by isoelectric precipitation and chromatography on carboxymethyl cellulose. The pH 6.5 kinase washes through the carboxymethyl cellulose column, while the pH 9.5 kinase is eluted by 0.2 M NaCl, 0.01 M Tris-HCl (pH 7.5).

Among the basic proteins examined, histone IV is phosphorylated most extensively, while histone IIb and protamine are slightly phosphorylated by the pH 9.5 kinase. Histone I, casein, bovine serum albumin, poly-L-lysine, and poly-L-arginine are only poorly or not at all phosphorylated (Fig. 2a). The pH 6.5 kinase most extensively phosphorylates histone I, while histone IV is phosphorylated to a lesser extent (Fig. 2b). The pH 9.5 and pH 6.5 kinases are further distinguishable from one another with respect to their kinetics of phosphate incorporation, which may be related to the behaviour of histone IV in solution at pH 9.5.

When 3H -histone IV (isolated by the Johns method¹⁴ from Walker-256 carcinosarcoma cells grown in tissue culture in medium containing 3H -arginine) was phosphorylated using the pH 9.5 kinase and isolated by electrophoresis on cellulose acetate¹⁵ for 15 min at 110 V, the electrophoretic mobility, as measured by the co-migration of the 3H and the ^{32}P labels toward the anode, was altered significantly from that of unphosphorylated 3H -histone IV. The latter migrates very slowly toward the cathode under these conditions. Analogous studies using 3H -histone I and the pH 6.5 kinase resulted in a similar alteration in the mobility of histone I. Dephosphorylation of histone I phosphate yielded free ^{32}P -phosphate and intact histone I.

Hydrolysis in 3 M KOH of the ^{32}P -labelled histone IV result-

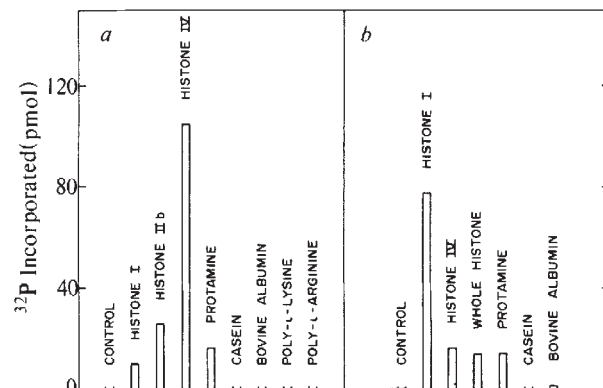


Fig. 2 Substrate specificity of the partially purified histone kinases. a, pH 9.5 kinase; b, pH 6.5 kinase. 20 μ g of the specified protein was added to the reaction mixture for each determination and assays performed as outlined in the text.

ing from the pH 9.5 kinase and subsequent paper chromatography of the hydrolysate in *n*-propanol:ethanol: $H_2O:NH_3$ (30:30:39:1) yielded ^{32}P -3-phosphohistidine. This observation has been confirmed by coupled Pronase-trypsin digestion of the phosphorylated histone IV. Studies on the chemistry of the phosphorylated lysine-rich histone produced by the pH 6.5 kinase reaction are in progress.

Our study shows the existence of at least two previously undiscovered nuclear histone kinases which catalyse the formation of acid-labile histone phosphates. Previously, the only known histone phosphates contained acid-stable phosphomonoester linkages. The two histone kinases we have found are distinguishable from one another with respect to various criteria, including the optimum pH for activity and the type of histone phosphorylated.

It is generally believed that phosphorylation of histones results in a loosening of the interaction between histones and DNA so that transcription or replication can occur. The temporal relationship of the new histone kinases to DNA synthesis and other cell cycle events is being studied with synchronised Walker-256 carcinosarcoma cells in culture.

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DONALD L. SMITH
BERNDT B. BRUEGGER
RICHARD M. HALPERN
ROBERTS A. SMITH

Department of Chemistry and Molecular Biology Institute,
University of California, Los Angeles, California 90024

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- Ord, M. G., and Stocken, L. A., *Biochem. J.*, **98**, 5P (1966).
- Kleinsmith, L. J., Allfrey, V. G., and Mirsky, A. E., *Proc. natn. Acad. Sci. U.S.A.*, **55**, 1182 (1966).
- Sung, M., Dixon, G. H., and Smithies, O., *J. biol. Chem.*, **246**, 1358 (1971).
- Hayashi, T., and Iwai, K., *J. Biochem. Tokyo*, **68**, 415 (1970).
- Langan, T. A., *J. biol. Chem.*, **244**, 5763 (1969).
- Cross, M. E., and Ord, M. G., *Biochem. J.*, **118**, 191 (1970).
- Sung, M., and Dixon, G. H., *Proc. natn. Acad. Sci. U.S.A.*, **67**, 1616 (1970).
- Langan, T. A., *Science*, **162**, 579 (1968).
- Boyer, P. D., and Bieber, L. L., in *Methods in Enzymology* (edit. by Estabrook, R. W., and Pullman, M. E.), **10**, 768 (Academic Press, New York, 1967).
- Chauveau, J., Moule, Y., and Rouiller, C., *Expl Cell Res.*, **11**, 317 (1956).
- Lovtrup-Rein, H., and McEwen, B. S., *J. Cell Biol.*, **30**, 405 (1966).
- Higgins, G. M., and Anderson, R. M., *Arch. Path.*, **12**, 186 (1931).
- Wang, T. Y., *J. biol. Chem.*, **242**, 1220 (1967).
- Johns, E. W., *Biochem. J.*, **92**, 55 (1964).
- Machicao, F., and Sonnenbichler, J., *Biochim. biophys. Acta*, **236**, 360 (1971).

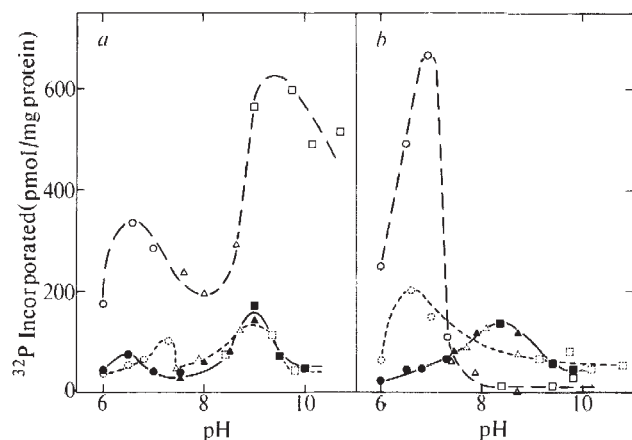


Fig. 1 Acid-labile histone phosphate produced as a function of pH using nuclear extracts. a: — — —, Walker-256 carcinosarcoma; - - -, rat spleen; — — —, regenerated rat liver. b: — — —, rat brain; - - -, rat liver; — — —, rat kidney. Buffers used were: ○, ○, or ●, phosphate (Walker-256 carcinosarcoma and rat liver) or maleate (other tissues); △, △, or ▲, Tris-HCl; □, □, or ■, glycine-NaOH.

Elevated Erythrocyte Calcium in Sick Cell Disease

CUMULATIVE membrane damage and loss is thought to occur in the erythrocytes of individuals homozygous for haemoglobin S (refs 1-4). The culmination of this process seems to be the formation of irreversibly sickled cells (ISC), which will not resume normal biconcave disk shape, even when the haemoglobin which they contain is in the sol (oxygenated) state⁵⁻⁷. Although the processes responsible for the formation of ISC have not yet been fully elucidated, it is clear that a change in the structure or composition of the membrane has occurred, such that cellular plasticity and ability to resume normal shape upon oxygenation have been lost. Weed *et al.*⁸ report a similar loss of plasticity, a diminished ease of ingress into glass microcapillaries, when calcium is placed inside resealed membrane "ghosts" made from normal human erythrocytes. In whole red cells, diminished filterability was also found to occur concomitantly with progressive loss of ATP. ATP-depleted red cells, however, retained most of their plasticity if EDTA were added to the incubation mixture to prevent calcium accumulation⁸. These results indicate that increased calcium alone may markedly reduce red cell plasticity.

From this evidence, and the fact that sickle cells, especially in the deoxygenated, sickled state, are excessively permeable to cations such as sodium and potassium⁹⁻¹¹, we postulated that increased intra-erythrocytic calcium concentrations might occur in Hb SS disease. This accumulation of calcium within the red cell might lead to diminished filterability, and, eventually, to the formation of ISC. The results reported here support this hypothesis.

Erythrocytes from heparinized blood samples were washed four times in phosphate buffered glucose saline (pH 7.40, 10 mM dextrose) before quantitative analysis of red cell calcium concentration. This was carried out by atomic absorption spectroscopy according to the techniques reported by Weed *et al.*³ except that only one trichloroacetic acid (TCA) extract was made, one volume of thoroughly washed red cells being precipitated with five volumes of cold 10% TCA with 20,000 p.p.m. lanthanum. Quantitative recovery using this extraction technique was confirmed by counting extracts of erythrocytes which had been previously equilibrated with $^{45}\text{Ca}^{2+}$. Scanning electron microscopy and energy dispersive X-ray spectroscopy were performed on erythrocytes mounted on glass cover slips, fixed in 1% glutaraldehyde and subsequently washed in calcium-free isotonic dextrose to remove surface salt contamination. Individual ISC, defined as erythrocytes deviating markedly from biconcave disk shape, and sickle erythrocytes of normal morphology were found using scanning electron microscopy (Kent-Cambridge Stereoscan microscope). Energy dispersive X-ray spectroscopy¹² was used to analyse these individual cells for calcium. This technique involves exposing portions of individual cells to an electron source and measuring the resultant dispersed energy arising from individual elements within the cell. Because this technique does not allow absolute quantitative analysis, calcium was expressed as the ratio of calcium counts to aluminium counts. The aluminium counts were simultaneously obtained from the collector face of a beryllium-windowed 'Keve' X-ray detector. Calcium flux studies were performed using washed packed red cells suspended to 30% by volume in Krebs-Ringer bicarbonate, containing 10 mM dextrose, 2.5 mM CaCl_2 , and 5.0 $\mu\text{Ci ml}^{-1}$ $^{45}\text{Ca}^{2+}$. After incubation with labelled calcium, the red cells were thoroughly washed at 4° C to remove all external calcium, extracted with five volumes of 6.7% TCA and the residual radioactivity was counted. In all procedures, as many white cells as possible were removed.

Quantitative analysis of erythrocyte calcium by atomic absorption spectroscopy revealed that Hb SS erythrocytes contained approximately eight times more calcium than erythrocytes from normal controls (Table 1). Furthermore,

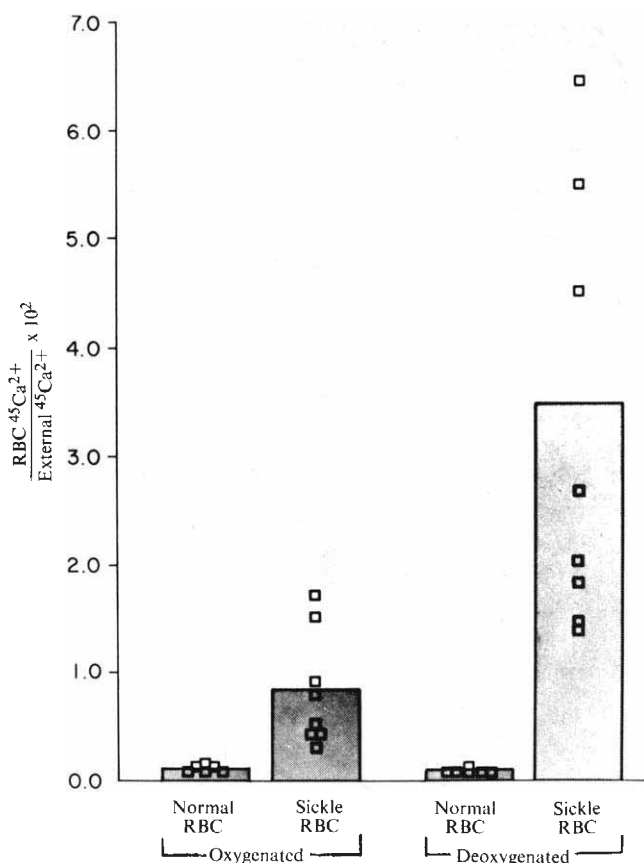


Fig. 1 Calcium leak into oxygenated and deoxygenated normal and sickle erythrocytes expressed as % of external $^{45}\text{Ca}^{2+}$ entering the red cells during 1.5 h incubation in a shaking water bath at 37° C. Samples of washed red cells were equilibrated in a tonometer with either 95% O_2 and 5% CO_2 or 95% N_2 and 5% CO_2 . □, Individual values. Means for each group are represented by the bars. Means (±1 s.d.) are as follows: oxygenated normal RBC, 0.090 ± 0.035 ; deoxygenated normal RBC, 0.072 ± 0.028 ; oxygenated sickle RBC, 0.840 ± 0.572 ; deoxygenated sickle RBC, 3.490 ± 1.884 . Significance of differences (Student's *t* test, two tailed): between oxygenated normal and sickle RBC, $t=3.177$, $P<0.01$; between deoxygenated normal and sickle RBC, $t=4.398$, $P<0.001$; between oxygenated and deoxygenated sickle RBC, $t=3.808$, $P<0.01$.

energy dispersive X-ray spectroscopy of individual Hb SS erythrocytes showed that, within the same preparation of well-oxygenated red cells, those which were "irreversibly sickled" contained approximately twice as much calcium as red cells of normal morphology. The ratio of calcium to aluminium averaged 3.01×10^{-3} (± 0.68) in eight ISC compared to 1.52×10^{-3} (± 0.04) in five sickle red cells of normal morphology ($t=4.839$, $P<0.001$).

To determine the origin of the greatly increased calcium concentrations, the influx of $^{45}\text{Ca}^{2+}$ into Hb SS red cells was measured. When washed red cells were incubated in Krebs-Ringer bicarbonate buffer with $^{45}\text{Ca}^{2+}$, the net influx of calcium into fully oxygenated Hb SS red cells was almost ten times that into normal red cells (Fig. 1). Furthermore, when oxygenated and deoxygenated (sickled) Hb SS erythrocytes were compared, it was found that calcium flux was enhanced

Table 1 Erythrocyte Calcium Concentrations in Normal Subjects and Patients with Haemoglobin SS Disease

Subjects	n	Mean	Range
Normal	14	24.9 (±8.2)	15.5-38.9
Hb SS disease	9	188.8 (±68.8)*	111.0-302.3

Values are expressed in $\mu\text{mol Ca}^{2+} \text{ l}^{-1}$ packed red cells ± 1 s.d.
* $t=8.824$, $P<0.001$ (Student's *t* test, two tailed).

more than four-fold by the sickling of Hb SS red cells. In contrast, deoxygenation had no effect on calcium leak into normal red cells. Control studies on samples from patients with elevated (>7%) reticulocyte counts revealed normal calcium flux rates (data not shown). Most of the uptake of $^{45}\text{Ca}^{2+}$ by sickle red cells probably represents exchange of unlabelled intracellular calcium for labelled extracellular calcium but three samples of deoxygenated Hb SS red cells, incubated for 1.5 h at 37° C, were found to accumulate an average of 50 $\mu\text{M Ca}^{2+}$ l^{-1} of packed cells. No net accumulation was detectable in three control samples from normal individuals. This calcium accumulation in Hb SS erythrocytes was equivalent to a 25% increase in total cellular calcium.

We have found that Hb SS erythrocytes contain approximately eight times as much calcium as do erythrocytes from normal individuals, that ISC appear to contain approximately twice as much calcium as do Hb SS cells of normal morphology and that Hb SS red cells display markedly increased permeability to calcium, which is further enhanced by deoxygenation and sickling of these erythrocytes. This evidence indicates that the leakiness of Hb SS red cells leads to an abnormal accumulation of intracellular calcium. This, perhaps combined with progressive metabolic depletion, could explain the diminished filterability of oxygenated Hb SS red cells which has been reported by several investigators¹³⁻¹⁶, and is likely to be critically involved in the shortened survival of Hb SS red cells. LaCelle *et al.*¹⁷ have proposed that increased calcium and decreased ATP concentrations cause the stiffening of the erythrocyte membrane which characterizes normal senescent red cells. Membrane stiffening, in turn, would lead to sequestration and destruction of erythrocytes in reticuloendothelial organs such as the spleen and liver. A similar, though much more rapid, series of events seems to underlie the greatly accelerated red cell destruction in haemoglobin SS disease. In addition, marked calcium accumulation may be involved in the formation of ISC *in vivo*.

We suggest, as have others⁸, that calcium alters the sol-gel characteristics of the red blood cell membrane, favouring the latter. In Hb SS erythrocytes, an excessive accumulation of membrane calcium, occurring during sickling, probably "fixes" the membrane in the sickled shape, thereby producing the ISC.

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JOHN W. EATON

Department of Medicine,

T. D. SKELTON

Bioengineering Research Group,

Department of Mechanical Engineering,

HAROLD S. SWOFFORD

CHARLES E. KOLPIN

Department of Chemistry,

HARRY S. JACOB

Department of Medicine, University of Minnesota,
Minneapolis, Minnesota 55455

Received July 23, 1973.

- ¹ Jensen, W. N., Bromberg, P. A., and Bessis, M. C., *Science N.Y.*, **155**, 704 (1967).
- ² Padilla, F., Bromberg, P. A., and Jensen, W. N., *J. Lab. clin. Med.*, **72**, 1000 (1968).
- ³ Jensen, W. N., *Am. J. med. Sci.*, **257**, 355 (1969).
- ⁴ Jensen, W. N., Bromberg, P. A., and Barefield, K., *Clin. Res.*, **17**, 464 (1969).
- ⁵ Döbler, J., and Bertles, J. F., *J. exp. Med.*, **127**, 711 (1968).
- ⁶ Bertles, J. F., and Milner, P. F. A., *J. clin. Invest.*, **47**, 1731 (1968).
- ⁷ Bertles, J. F., and Döbler, J., *Blood*, **33**, 884 (1969).
- ⁸ Weed, R. I., LaCelle, P. L., and Merrill, E. W., *J. clin. Invest.*, **48**, 795 (1969).
- ⁹ Tosteson, D. C., Shea, E., and Darlin, R. C., *J. clin. Invest.*, **31**, 406 (1952).
- ¹⁰ Tosteson, D. C., Carlsen, E., and Dunham, E. T., *J. gen. Physiol.*, **39**, 31 (1955).
- ¹¹ Tosteson, D. C., *J. gen. Physiol.*, **39**, 55 (1955).

- ¹² Reviewed in Bertin, L. P., *Principles and Practice of X-Ray Spectroscopic Analysis* (Plenum Press, New York, 1970).
- ¹³ Jandl, J. H., Simmons, R. L., and Castle, W. B., *Blood*, **18**, 133 (1961).
- ¹⁴ Charache, S., and Conley, C. L., *Blood*, **24**, 25 (1964).
- ¹⁵ Dintenfass, L., *J. Lab. clin. Med.*, **64**, 594 (1964).
- ¹⁶ Schmid-Schönbein, H., and Wells, R., in *Abstr. Int. Soc. Hemorheology, 2nd Int. Conf., Heidelberg, Germ.*, 25 (1969).
- ¹⁷ LaCelle, P. L., Kirkpatrick, F. H., and Udkow, M., in *Erythrocytes, Thrombocytes, Leukocytes* (edit. by Gerlach, E., Moser, K., Deutsch, E., and Wilmanns, W.) (Georg Thieme, Stuttgart, 1973).

Erratum

IN the article "Accumulation of Fossil CO₂ in the Atmosphere and the Sea" by A. W. Fairhall (*Nature*, **245**, 20; 1973), Figs 1 and 2 of the original manuscript were substituted for the corresponding figures of the revised version. As far as Fig. 2 is concerned, the differences are small, but Fig. 1 (revised) contained data which were essential to the discussion. The correct figure appears below. Also line 2 of page 22 should read, " $[n_a^{t'} - n_a]/[N_a + n_a(t')]$, expressed as a percentage where $t' = "$ ".

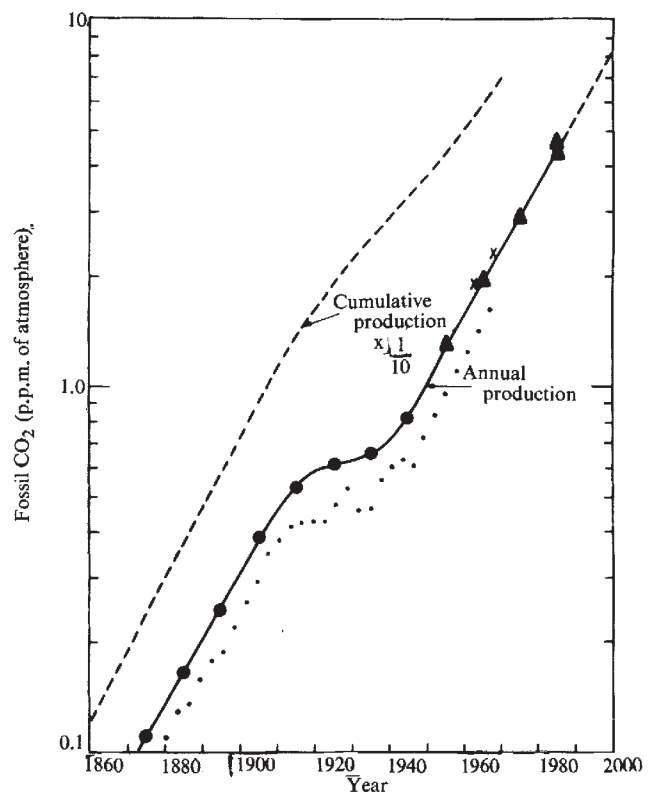


Fig. 1 Production of fossil CO₂ since the 1870s: the solid circles are the estimates of Revelle and Suess¹⁰ extended to 1890 by estimates of OECD, which along with the data from the 1960s are from ref. 11. The dots are estimates of Keeling¹². The dashed line is the cumulative production to the present day based on the Revelle-Suess production curve.

The author also wishes to take this opportunity to add the following information:

Using a simple box model for the atmosphere, biosphere, mixed layer of the sea, and the deep sea, and a logistic-curve representation of future fossil CO₂ emissions, Zimen and Altenhein^{1,2} have reached substantially the same conclusions as myself. In their analysis the calcite undersaturation of the sea would be reached before the turn of the century.

¹ Zimen, K. E., and Altenhein, F. K., *Naturwissenschaften*, **4**, 198 (1973).

² Zimen, K. E., and Altenhein, F. K., *Z. Naturforsch.* (in the press).

BOOK REVIEWS

Plants People Ate

Palaeoethnobotany: The Prehistoric Food Plants of the Near East and Europe. By Jane M. Renfrew. Pp. xvii+248 (48 plates). (Methuen: London, May 1973.) £6.50.

DR RENFREW begins this attractively produced book with a definition of palaeoethnobotany: "the study of the remains of plants, cultivated or utilised by man in ancient times, which have survived in archaeological contexts". She is concerned primarily with the palaeoethnobotany of the Near East and Europe in prehistoric times and with plants utilised for food rather than as sources of structural materials, textiles, dyes or medicaments. Her main intention is to bring together in a single volume the information available for the identification of archaeological plant material, for without accurate identification the results of palaeoethnobotanical investigations cannot be interpreted meaningfully. The book therefore describes and assesses the evidence from which it has proved possible to attempt a reconstruction of the history of man's plant foods during the past ten thousand years or so.

The initial chapter reviews the development of palaeoethnobotanical research since the pioneering publications of C. Kunth (1826) and Oswald Heer (1866), and pays just tribute to the long series of fundamentally important contributions by Hans Helbaek, of the National Museum in Copenhagen, whose establishment of principles and techniques for the identification of plant remains and especially of seeds, grains and fruits has placed all workers in this field deeply in his debt.

There follows a general survey of the evidence, the survival of which has been for the most part due to carbonisation by heat, continuous waterlogging in peat deposits or desiccation in desert sand but also to the accidental preservation of impressions of seeds and fruits in the wet clay of pottery before firing or, less frequently, in the mudbrick or daub of walls before sun-drying. With regard to the very large numbers of grains and seeds found carbonised, Dr Renfrew gives no support to the belief that this might be the result of spontaneous anaerobic processes taking place at normal temperatures but regards carbonisation as arising simply from overheating near a hearth or in a parching oven or sometimes from a house fire: she reminds us that the ears of hulled wheats require parching before they can be successfully threshed or winnowed. She, like many others, has

been concerned about the effect of carbonisation, or indeed of any mode of preservation, on the dimensions and shape of plant parts and she quotes the results of experiments, including her own, which enable estimates to be made of the extent of deformation in various circumstances. It is of interest that prehistoric grains must in most instances have been smaller than their counterparts of today.

Carbonised material may preserve a good deal more than the gross morphological features: "occasionally the surface . . . is so well preserved that it is possible to study the cell pattern and even the structure of the hairs". Helbaek has shown, too, that silica skeletons of epidermal cells of cereal grains and their glumes may persist even in impressions in mudbrick or baked clay, thus much increasing the possibilities of critical identification. It seems likely that the services of the scanning electron microscope will become increasingly valuable in future.

The palaeoethnobotanist seeks to answer such questions as what type of food economy was practised and, if there was crop agriculture, what were the crops, whether they were grown singly or in mixture and in what relative amounts. This demands not only the discovery of suitable sites but also the correct decisions as to how the plant remains are to be extracted and sampled. Dr Renfrew shows in a table of results from Tell Azmak in Bulgaria that the whole of the material in a single find may constitute too small a sample for any but very hazardous inferences: finds at contemporary levels in the same site have remains of from one to five plant species. Even where there is adequate material there are dangers of sampling bias from various causes, and the finds themselves are likely to be products of some degree of contemporary selection, so that interpretation must be doubly cautious. The whole topic is of the utmost importance and it could be argued that Dr Renfrew might have devoted even more space to it.

Chapters 4 to 16 describe the major temperate food plants of prehistoric sites, indicating the location and dating of their finds and explaining how they can be identified and differentiated from their closest relatives. There are many excellent line drawings by Alan Eade and some informative half-tone plates at the back of the book. There are also numerous tables of characters and measurements, so that palaeoethnobotanists should be greatly assisted in solving problems of identification.

Chapter 20 considers the food values of prehistoric plant foods, but it is difficult to infer more than that the foods included important sources of carbohydrates, fats and proteins and that various fruits were probably eaten fresh: green foods would not have been preserved. The final chapter is a brief review of the origins and development of crop agriculture in the Near East and its spread to Europe. Dr Renfrew's own investigations in Europe give great weight to her views on this topic. She accepts that the first farmers reached the Balkan Peninsula soon after 6000 BC and that they brought emmer wheat as the chief cereal crop, together with einkorn wheat, both two-row and six-row barleys, oats and various pulses. The naked club and bread wheats appear quite early in the neolithic, but spelt wheat and rye not before the late neolithic. Dr Renfrew is rightly content to present the archaeological facts and does not much concern herself with evolutionary theories based on cytogenetic findings. A much larger body of archaeological data seems necessary before workers should be worried by any failures of the two lines of approach to yield identical conclusions.

Dr Renfrew emphasises the antiquity and scale of the cultivation of leguminous crops. Peas, lentils and vetches found in the earliest farming sites may have been collected in part from wild plants, but the cultivation of pulses seems as old as that of cereals. Much of the general interest of this book lies in the information provided about food plants other than cereals and pulses. Thus acorns, pistachio nuts, cornelian cherries (*Cornus mas*) and crab apples occur in early neolithic farming sites in SE Europe, and figs, wild pears and wild grapes in Greece from the late neolithic onwards. Further north there is evidence for the collection or cultivation of cherries, bullaces, sloes, raspberries, blackberries, haws, whitebeam fruits, rose hips, elderberries, bilberries and water chestnuts (*Trapa natans*). Besides acorns there are almonds, walnuts, hazelnuts and beech-nuts; and there are the numerous weed seeds which are known to have been collected, some more and some less intentionally, and eaten in bread or porridge. The whole list is interesting and impressive as illustrating the large-scale persistence of food collecting into the agricultural period.

There are a few misprints and a few obscurities, and Helbaek's 1963 publication, cited on pages 9 and 11, does not appear in the bibliography. These are small matters. Dr Renfrew must be

congratulated on having achieved her aim of providing for palaeoethnobotanists a much more comprehensive and systematic guide to the identification of plant remains than has been available until now and, moreover, of providing at the same time a very welcome source book of information for the general scientific reader.

A. R. CLAPHAM

The Excitable Heart

Electrical Phenomena in the Heart. Edited by Walmor C. De Mello. Pp. xv+415. (Academic: New York and London, October 1972.) \$26.

New blood has kept the field of cardiac electrophysiology fertile and by drawing upon it Professor De Mello has ensured the success of his enterprise, although the absence of a preface is a pity. Those starting a career in cellular physiology and biophysics, and to whom the book is addressed, need to know how the editor views the subject as a whole: better to expose the logic or hunch by which the chapters have been strung together than to imply questionable connexions by tacit juxtaposition.

The volume opens with "Electrical Properties of Embryonic Heart Cells" by N. Sperelakis. From the biological point of view this is a rational beginning, but since familiarity with the electrophysiology of the adult tissue is required for the appreciation of this informative article, some readers will find W. K. Berger's chapter on "Ultrastructure and Function of Cellular Contacts" an easier starting point. The morphology of cardiac muscle and the mechanism of excitation spread are well reviewed therein. H. Fozzard's lucid article on membrane capacity of cardiac muscle cells, out of place as chapter 8, might well be read next since structural considerations enter closely into the interpretation of the measurements.

The task of exposing the nature of the cardiac action potential is shared out between J. Dudel who deals mainly with the fast excitation process, and D. Noble and R. W. Tsien who cover the events during the plateau phase in a helpfully interpretative way. The generation of action potentials in spontaneously beating Purkinje and auricle fibres is touched upon briefly by both Dudel and Noble and Tsien. The reader pursuing the leads to voltage-clamp experiments given in their articles will find that the pacemaker potential can also be ascribed to voltage and time-dependent conductance changes controlling the downhill flow of ions. Quite a different hypothesis is advanced by T. C. West who, in writing on "The Electrophysiology of the Sinoatrial Node", holds the main brief to deal with cardiac autorhythmicity. On the basis

mainly of the high temperature coefficient of the heart rate, he implicates active inward transport of potassium ions in the fall of K permeability during the diastolic interval. As an alternative hypothesis this may well require consideration, but it is puzzling why the voltage-clamp experiments bearing on the problem of autorhythmicity have been totally ignored. Presumably the editor was left to turn diverse views to constructive ends; but in the event this task falls upon the reader. From the touchlines the editor does, however, bias the issue by setting H. G. Haas's article on "Active Ion Transport" immediately before that on the sinoatrial node, as if the consideration of active transport were an essential preliminary to the problem of autorhythmicity.

Taken by itself the chapter on active transport makes rewarding reading. The emphasis placed on the great sensitivity of the cardiac action potential to variations in the metabolic state of the cells is well placed as this remains a pressing problem. The case for the participation of metabolically driven currents in shaping the action potential is, however, not compelling. A less direct mechanism, say a change in the intracellular concentration of an ion determining a surface potential on the inner face of the excitable membrane, could equally well be at work.

Under the title "Cardiac Innervation and Synaptic Transmission" M. Anderson and J. del Castillo cover the recent revival of histological research into the rich innervation of the specialized regions of the heart. The high chemosensitivity of natural pacemaker fibres permitted some time ago the demonstration of the mode of action of acetylcholine. The more recent work on the inotropic action of adrenaline is well summarized, but the treatment of the chronotropic action suffers from the inadequate cover given earlier in the book to the known kinetics of the pacemaker current.

From a perceptive chapter on atrioventricular transmission by C. Mendez and G. R. Moe it becomes clear that the slow inward current system plays an important part in the propagation of excitation through the node. At this point one misses in this otherwise laudable multinational book a contribution from one of the French laboratories where the slow inward current system has been explored.

After interesting chapters on "Comparative Aspects of Electrogenesis" by F. V. McCann and on "The Healing-Over Process" by W. C. De Mello the volume is rounded off with a comprehensive article on "Calcium Movements and Excitation-Contraction Coupling" by J. B. Bassingthwaite and H. Reuter.

O. F. HUTTER

Feeding Children

Malnutrition and Retarded Human Development. By S. L. Manocha. Pp. xv+382. (Charles C. Thomas: Springfield, Illinois, September 1972.) \$19.75.

WE have known since the early years of this century that good nutrition has something to do with physical and mental development, and in many countries practical schemes to feed growing children as well as possible have been introduced. During the past twenty years or so during which international concern over the world food situation has intensified there has been increasing effort to solve questions relating to the nutritional needs of children, with the general aim that children everywhere shall be adequately nourished.

Many scientific problems remain to be solved, but much is now known about the nutritional needs of children and how to meet them, and Dr Manocha has done a useful service in assembling so much material in this review. His book, which is intended for the educated layman as well as the professional reader, is clearly and simply written. He discusses the biochemistry and physiology of human development, particularly of the brain, environmental influences, including socio-economic factors, maternal nutrition and food habits, and such practical matters as government schemes to alleviate malnutrition, guidance on the direction and treatment of the diseases of protein-energy malnutrition, and nutrition education.

Dr Manocha writes from an international point of view and shows by the number and variety of the references he cites that he has crossed national boundaries and brought together relevant material from established and active workers in all continents. Each of the nine chapters of the work ends with a list of authoritative references ranging in time from the early years of international anxiety about malnutrition in children to 1971. These lists would by themselves be invaluable to any student of nutrition.

One could point to minor errors in the names of certain authors and to the omission of ideas that appear to be becoming of crucial importance, such as how the overriding socio-economic problems of modern society are to be solved if proper physical and mental development is ever to be achieved for all human beings. But to do this would be to carp. This book can be recommended as a useful, comprehensive, simply written review of an important and topical subject. It could be read with profit by anyone who wishes to understand more about one of the most intractable subjects of our age.

D. F. HOLLINGSWORTH

Brown on Boranes

Boranes in Organic Chemistry. By Herbert C. Brown. Pp. xiv+462. (Cornell University: New York, August 1972.) \$24.50.

THIS book is part of a distinguished series in which eminent chemists have provided written records of their Baker Lectures at Cornell University. Professor Brown essentially gives a scientific autobiography. In this sense the title of the book is misleading in that the subject of organoboranes forms but one of six parts discussing various aspects of borane chemistry.

In part 1 the principal topics relate to diborane and borohydrides, and free radical substitution reactions. These two diverse topics commanded Professor Brown's interest because of the presence at the University of Chicago of H. I. Schlesinger and M. S. Karasch.

Part 2 is devoted to Brown's contribution to the assessment of the role of steric strains in chemistry. His work on donor-acceptor addition complexes of boron led him to the idea of these as models for saturated carbon compounds—strained homomorphs and the classification of steric strains (F-, B-, I-strains).

The non-classical ion problem, which is the topic of part 3, has been the subject of long-standing controversy, particularly with the late Saul Winstein, and has now become a part of chemical folklore. The relationship to the boron studies is perhaps based on the tenuous recognition that a carbonium ion is iso-electronic with a borane.

Part 4 is concerned with selective reductions using hydrides or complex hydrides of boron and aluminium and the question of selectivity. Reagents such as disiamylborane are uniquely associated with Brown, not least in their nomenclature.

In part 5, which is devoted to hydroboration and essentially updates Brown's monograph on the subject of 1962, the author is mainly concerned with boron-carbon bond-making. The term "hydroboration" is probably interpreted by the organic chemist as a combination of this process together with a subsequent boron-carbon bond-making, usually without isolation of the boron intermediate. The value of the overall procedure lies in a combination of factors which includes mild reaction conditions, essentially quantitative yields, a high degree of selectivity and stereospecificity, and a multiplicity of similar specific cleavage reactions. Thus it is possible to convert acetylenes or olefins to numerous functional derivatives including higher homologues. Part 6 deals with organoboranes from the point of view of boron-carbon cleavage reactions. From the standpoint of the

organic chemist, parts 5 and 6 thus form a continuous story.

Professor Brown is, and has been, one of the giants of chemistry of the last thirty years. He is a splendid controversialist, both verbally and in writing, and the present contribution is in this same happy tradition. M. F. LAPPERT

Structures in Cells

The Generation of Subcellular Structures. By R. Markham, J. B. Bancroft, D. R. Davies, D. A. Hopwood and R. W. Horne. Pp. x+372. (North-Holland: Amsterdam and London; Elsevier: New York, 1973.) Dfl 45; £7.

THE three-dimensional structures of subcellular biological systems and mechanisms for their biosynthesis are now elements of perhaps the most important next stage in molecular biology. The problem is ripe for attack and the timing and choice of this subject for the first John Innes Symposium (held in July 1972) could not have been more appropriate. It has been excellently organized and the individual subsections well selected, being here presented in a series of stimulating articles by leading exponents from many parts of the world.

Prefaced by a suitably general historical review of molecular genetics in the form of the sixth Bateson Memorial Lectures by W. Hayes, the proceedings of the meeting include a fairly wide collection of original reports on the structure and synthesis of organelles (usually in the form of summaries of recent advances). The range stretches from relatively simple systems such as bacterial flagella and plant viruses through larger viruses, such as T₄ phage, towards bacterial spores and the more complex sophistications of ribosomes, cell walls and mitochondria. Contributors include D. Kerridge, H. R. Revel, E. Kellenberger, J. G. Atabekov, M. Nomura, R. A. Cox, A. Ryter, P. C. Fitzjames, Audrey Glauert, B. A. Afzelius and many others.

It is worth noting that all the systems considered here derive from viruses or prokaryotes, where the relatively simple structures involved are easier to study than the complexities of eukaryotes, but where the basic lessons to be learnt and the models that emerge are applicable to all types of living systems.

Perhaps the most fundamental problem in this field is the extent to which all necessary information for the correct assembly of subcellular organelles (indeed for the whole cell, for that matter) is contained in the structures of their constituent molecules. Another (slightly more restricted) way of putting it would be to ask whether the DNA or RNA genome contains all the necessary instructions for putting together the nor-

mal three-dimensional structures involved. This naturally leads to two rather more specific queries. (1) How far can assembly of multimolecular complexes occur in the absence of pre-existing structure and, in cases where some sort of pre-existing structure is required, how specific must it be? (2) To what extent, in the construction of such complexes, is a sort of scaffolding necessary—in the sense of an essential structure that is not contained in the final finished organelle?

The articles in this symposium do not of course provide a complete, generalized answer to this sort of question. But they begin to indicate the sorts of ways in which answers can be given. It would be impossible to summarize any general conclusions, although the reader is left with a strong feeling that this is an area where much progress will occur rapidly during the next 10 years.

Perhaps, above all, what has emerged fairly clearly is the extent to which spontaneous assembly of relatively complex structures can occur (being often demonstrable *in vitro*) simply through molecules "finding each other" by interactions between complementary patterns on their surfaces. Temporary scaffolding, specific catalysis by enzymes, complex "foundation" molecules acting as a specific nidus for the association of other key molecules—if they exist (as they appear to do in certain cases)—are not always necessary, although there is often an obligatory sequence in the assembly process (for example, with ribosomes) and/or a polarity in the direction of build-up, even of monomers in simple complexes such as flagellar filaments.

Having emphasized what a fascinating collection of articles is here available in an exciting field, some protest must be made about the publication itself. The offset-type process used is more tiring and difficult to read than ordinary print, the legends are not properly distinguished from the text, some tables are in type almost too small to decipher, the section headings do not stand out clearly and italics are not properly visible. The discomfort involved might be tolerable if the book were cheap.

M. R. POLLOCK

Practical Concern

Guide for Safety in the Chemical Laboratory. Second edition. (Manufacturing Chemists Association.) Pp. vi+505. (Van Nostrand Reinhold: New York and London, 1972.) £8.75.

A SMALL stretch of the imagination should inform us that the first task of the contemporary, socially concerned scientist is to make sure that neither he nor those working with him are blown up, burnt to cinders or dissolved in acid. Yet it is a matter of record

that very few institutions of higher learning are giving their students anything but the most cursory advice in laboratory safety and accident prevention on the time honoured basis that God, somehow, will couple the shorn lamb with the careless chemist. Industry, whose managers tend to be better informed on the hard realities of economic loss and the trade unions' inclination to fight compensation suits, is understandably more advanced.

In this situation it is always pleasant to welcome a new book which advocates more thought in the design and manning procedures of laboratories. Such publications come in two types: one, for example the *Handbook of Laboratory Hazards*, now published by The Chemical Society, lays emphasis on what to do once the accident has occurred and notes those chemicals with which potential danger is always present. The volume under review, produced by the American Manufacturing Chemists Association, takes a step back and considers safety as a logical outcome of sensible design and behaviour. Except in the most general sense it is not suitable as a quick reference volume in moments of stress: although it abounds in tables, so it does in cross references which take some time to follow through. But as background reading it is most valuable, for it takes nothing for granted and leads the teacher or laboratory supervisor step by well illustrated step through all the pitfalls of space, instruments and chemicals—when did you last test your fire extinguishers?

Frequently American practice differs from the British and there are variations in detail in regulations and materials standards: hence reference to ASTM. Nevertheless those whose work involves the manipulation of chemicals and the safety of the people concerned will derive great benefit from keeping *Guide for Safety* on their bookshelves, especially in the unlikely event that a fit of generosity from UGC, DES or head office allows them to translate dreams of a new laboratory into reality.

PETER FARAGO

Particle Physics

Elementary Processes at High Energy. ("Ettore Majorana" International Centre for Scientific Culture, 1970 International School of Subnuclear Physics, a NATO-MRI-MRST Advanced Study Institute sponsored by the Regional Sicilian Government and the Weizmann Institute of Science, Erice, July 1–19.) Edited by A. Zichichi. Part A. Pp. xix+1–448. Part B. Pp. xvii+449–861. (Academic: New York and London, April 1972.) \$29.50 each part.

THIS two-volume record of the 1970 International School of Subnuclear

Physics held at the hill-top town of Erice in Sicily, is a welcome addition to the scientific literature on elementary particle physics. It maintains a very high standard, and contains many lectures and discussions with permanent value.

Volume A contains the lecture courses and the review lectures, the relevant discussions being collected to follow each set. The lecture courses were of course quite topical. The opening lecture, "Electron-positron annihilation", by N. Cabibbo, was especially timely, in view of the recent operation of the electron-positron storage ring ADONE at Frascati and the early experimental results reported at the School by Italian groups working with ADONE.

G. Veneziano contributed an outstanding set of lectures about narrow resonance models compatible with duality. Besides his illuminating discussion of the origination of the model, he described clearly its generalisation to the N-point functions which many authors have discussed for scalar particles in the literature. T. D. Lee lectured on the Lee-Wick formulation of quantum electrodynamics, which makes use of an indefinite metric; his lectures included a critical analysis of the notion of causality, as used in elementary particle physics. H. Harari presented the qualitatively successful picture of hadron reaction processes which has resulted from an amalgamation of the duality model, the absorption model, and the quark model. Here his lectures are compressed into a novel but effective format, a picture book approach consisting of twenty-nine figures, self-explanatory to some extent but each with a figure caption, and a number of remarks added by way of introduction and conclusion. Whereas Harari's "picture book" occupies twenty-two pages, the discussion following them goes on for a further thirty-four pages.

Two review lectures on gravitational topics were given by W. Thirring from an elementary particle physicist's viewpoint, "more than a sort of exercise in Riemann geometry for esoteric theoreticians; something more down to earth". His second lecture "A trip behind the Schwarzschild radius", would, perhaps, be entitled today "Inside a black hole". Once considered outside elementary particle physics, gravitational topics are now attracting and intriguing many people in this field. Two review lectures were also contributed by T. D. Lee, the first an interesting introduction to symmetry principles in physics, and the second a critical discussion of published derivations of the $\Delta I = 1/2$ rule from soft pion theorems. Coming from Frascati, G. Salvini surveyed the status of experiments on electron-positron

storage rings which bear on quantum electrodynamics, on the vector dominance model for photon interactions and on searches for new vector mesons. Finally, B. Maglic reviewed the problems of meson spectroscopy, including his proposal to study $\pi\pi$ and $\mu\mu$ collisions at low energy, using the self-colliding orbits of his "Preceptron" device.

The seminar talks are collected together in volume B, and cover a wide range of topics, not all of which can be mentioned here. J. Adams recounted the possibilities considered for the super-CERN accelerator. His words are interesting to read today, and make us realise how wise was the final decision, in relation to the boundary conditions which existed at that time. V. Weisskopf contributed an instructive seminar about nucleon structure, as seen in the light of the scaling law now established for inelastic scattering. The study of $\pi\pi$, $K\pi$ and $K\bar{K}$ interactions was covered thoroughly in an extensive series of seminars by P. Schlein. Especially appropriate at this School were the seminars contributed by M. Conversi and by A. Zichichi, concerning the work in progress by their groups at ADONE.

Many other seminars with much topical interest were also given, about the light-cone behaviour in quantum field theory, and its application to lepton-pair production, about vector meson dominance and deep inelastic electron-proton scattering, about the Pomanchuk theorem and the possible ways in which it may fail, about SU(4) symmetry and its merits with respect to weak and strong interactions, and about PCAC, among other topics. One topic unusual for such a school was that discussed by U. Amaldi, the developments in nuclear physics which have been achieved by using high-energy particles produced by energetic accelerators. He described particularly how the analysis of quasi-free collisions, processes of the type (p,2p), has provided our strongest evidence for the validity of shell-model wave functions for light nuclei. The closing lecture, an interesting and authoritative history of the development of our knowledge of the properties of the weak decay interactions, was given by T. D. Lee.

The extensive discussion after each lecture has been an outstanding feature of the Erice School, and this has been recorded in full in these volumes. The audience included many students at the postdoctoral level, as well as many senior visitors who were there to lecture or to give seminars. Consequently, this discussion was at quite a high level, and frequently very illuminating. The record of these discussions is one of the most valuable aspects of this book.

R. H. DALITZ

CORRESPONDENCE

CO₂ Pollution of the Sea

SIR,—A recent communication (*Nature*, **245**, 20; 1973) suggested that a slight increase in partial pressure of CO₂ would shift the HCO₃⁻ equilibrium system far enough to prevent secretion of shells in marine corals and molluscs.

The lower carboniferous shells were secreted when the partial pressure of CO₂ was more than 300 times current levels (before the carbon of the coal measures was produced by species which themselves required high partial pressures of CO₂ to grow so luxuriantly).

The two statements are incompatible although the chemistry seems equally valid in both cases. However, I have never seen a systematic approach to palaeoatmospheric analysis and have long wondered at the ability of species to proliferate in spite of using an oxygen combustion system, in an atmosphere rich in CO₂, with primitive respiratory systems and inefficient internal oxygen transport arrangements. Popular assumptions about the primitive atmosphere may be wrong, otherwise ethologists have problems they have neglected.

Yours faithfully,

G. A. WOOD

13 Clifton Park Road,
Davenport Park,
Stockport

Are Conferences Necessary?

SIR,—As a means of communication of scientific data and theory, are conferences worth the money and effort being spent on them? Many think not, but

before such established and prestigious activity can be changed it is scientifically proper that experiments should be done.

Recently an attempt was made to break the established pattern by having a meeting based on the following concepts: (1) that satisfactory communication can only be achieved if the participants are limited to those actively engaged in relevant experimental work and who can sit around one, albeit large conference table; (2) that this close interaction between participants be maintained away from the conference table; (3) that participants should arrive knowing in detail the topics to be discussed; (4) that experiments to resolve some of the controversial problems described in the papers should be made at the meeting; (5) that these experimental procedures should be able to be changed on-line at the suggestion of the participants.

The subject of the conference was "Event Related Slow Potentials of the Brain" and was concerned with the changes of the electroencephalogram associated with external stimuli and with voluntary and involuntary motor movements ranging from speech to spinal reflexes. Forty-five invitations were sent to active workers in the field. It was estimated that about 200 people would have attended an unrestricted conference. The meeting was held in a relatively inaccessible college in which most of the participants were accommodated. Most of the forty papers were pre-circulated and only discussion was allowed at the meeting. One and a half days were devoted to experiments performed in the research laboratory of this Institute and relayed by closed cir-

cuit television. There was no mixing or editing of pictures from the four cameras as in normal television procedure; all cameras were on all the time and thus gave considerably more information than if the audience were in the laboratory. A television camera and microphone in the auditorium permitted participants to communicate with the demonstrators.

The demonstrations were separated into five sessions representing five aspects of the congress theme. They were presented by selected groups of participants with the help of the Institute staff. A total of 12 h television transmission was achieved.

There was good interaction between experimenters and audience and on occasions the experiments were modified to clear a particular point in dispute. These were experiments in the true sense of the word—procedures adopted on chance of their succeeding. All participants were enthusiastic about these experiments.

This format for conferences appears to be of considerable value in the presentation of data and organisers of meetings are urged to reconsider their objectives and methods and to carry out the necessary experiments.

Yours faithfully,

R. COOPER

*Chairman, Organising Committee,
3rd International Congress on Event
Related Slow Potentials of the Brain,
and*

*Scientific Director,
Burden Neurological Institute,
Stapleton, Bristol*

Obituary

A. G. Sharov

THE prominent insect palaeontologist Dr Alexander Grigorievitch Sharov died suddenly in Moscow on July 10, 1973.

Dr Sharov was born in 1922 in the village of Lukino, Moscow Region. While still a schoolboy he developed an interest in entomology, collecting insects and observing their habits and behaviour. After finishing school in 1939, he entered the Biological Faculty of Moscow University and specialised in entomology. While an undergraduate he took part in expeditions to the Far

East, Transcaucasia and Central Asia, where he collected material and made many interesting observations on the local faunas.

In 1943 Sharov was enlisted in the army and his military service lasted until the end of the war. He returned to Moscow University in 1945 and completed work for his diploma. In his student days, as in his later scientific work, Sharov was interested in the problems of insect ontogeny and evolution. His postgraduate studies began in the Institute of Evolutionary Morphology in Moscow, under the guidance of such prominent scientists as Pro-

fessors D. M. Phedotov and M. S. Ghilarov. In 1950 he was awarded a doctorate for his thesis on the 'proto-metabolous' development of the *Thysanura* and its bearing on insect phylogeny.

During the next few years Sharov's interests turned to fossil insects, and in 1955 he moved to the Palaeontological Institute of the Academy of Sciences of the USSR. There he worked until his death on the palaeontology, phylogeny and evolution of insects. He took part in many expeditions, collecting much remarkable fossil insect material. One of his most important discoveries was

that of the new order Monura, which he considered to be primitive ectotrophic Apterygota.

Sharov's works on the fossil insects of the Kuznetsky Basin are well known and highly regarded by insect palaeontologists. Most of his new discoveries were of Orthopteroid insects, and in 1968 he published a monograph on the phylogeny of this group. This comprehensive study, which was published in an English edition in 1971, will no doubt be the standard work on this subject for many years to come.

Sharov published a total of sixty-four papers and books. As well as a first-class scientist, he was a friendly and charming person, and his loss will be felt deeply by his many friends and colleagues.

Announcements

University News

Professor D. Samuel, Professor of Physical Chemistry, Weitzman Institute, has been appointed a Visiting Professor in the Department of Zoology and Comparative Anatomy, University College London.

Appointments

Dr J. W. B. King has been appointed director of the **Agricultural Research Council's Animal Breeding Research Organisation**.

Mr R. E. Boote has been appointed director of the **Nature Conservancy Council**.

The full membership of the **Computer Board for Universities and Research Councils** is now **Professor D. J. Finney**,

Professor A. J. Brown, **Mr A. H. Duncan**, **Professor J. C. Gunn**, **Dr J. Howlett**, **Professor K. W. Morton**, **Professor H. H. Rosenbrock** and **Professor M. Wells**.

Miscellaneous

Dr Basil Bard, Managing Director of the **National Research Development Corporation**, has been awarded the **Gold Medal** of the **Licensing Executives Society**.

Erratum

IN the article "Comparison between Ice Age and Present General Circulations" by E. B. Kraus (*Nature*, **245**, 129; 1973), equation 1 should read $\theta_s = \theta \exp(rL/c_p T)$, and Table 1, line 4, should read $T_3(^{\circ}\text{C})$. Ref. 9 should also be changed to read Bonatti, E., and Gardner, S., *Nature*, **244**, 563 (1973).

Corrigendum

IN the article "Prostaglandin Production and Bone Resorption by Dental Cysts" by M. Harris, M. V. Jenkins, A. Bennett and M. R. Wills (*Nature*, **245**, 213; 1973), $\text{PGF}_{1\alpha}$, PGF_2 and PGF_3 should read $\text{PGF}_{1\alpha}$, $\text{PGF}_{2\alpha}$, and $\text{PGF}_{3\alpha}$ throughout.

International Meetings

November 21, **Symposium on Acylation** (The Assistant Secretary, Society of Chemical Industry, 14 Belgrave Square, London, SW1X 5AG).

December 5-6, **Food Hygiene and the European Economic Community** (Mr M. D. Ranken, BFMIRA, Leatherhead, Surrey).

December 6, **Feeding the Newborn: Comparative Problems in Animals and**

Man (Dr J. D. Sutton, National Institute in Dairying, Shinfield, Reading RG2 9AT).

December 6-7, **Physical Properties of Solids at High Pressure** (The Meetings Officer, The Institute of Physics, 47 Belgrave Square SW1X 8QX).

December 11, **The Penetration of Liquids into Solids** (The Assistant Secretary, Society of Chemical Industry, 14 Belgrave Square, London SW1X 8PS).

December 11, **Teaching of Physics in Electronics Courses** (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX).

December 14, **Glaucoma Symposium** (Office of the Director, The University of Texas Health Science Center at Houston, Division of Continuing Education, PO Box 20367, Houston, Texas 77025).

December 17-19, **Computers in Spectroscopy** (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX).

December 17-21, **Congress on Marine Waste Disposal** (Professor E. F. Frangipane, Institute of Sanitary Engineering, Milan Polytechnic, Piazza Leonardo da Vinci 32, 20133, Milan, Italy).

December 18, **Recent Developments in Antibiotics** (Dr J. F. Cavalla, John Wyeth and Brother Ltd, Huntercombe Lane South, Taplow, Maidenhead, Berks).

December 18-19, **Polysaccharides: their Physical Chemistry and Molecular Biology** (Dr D. A. Rees, Unilever Research, Port Sunlight, Cheshire).

December 26-31, **American Association for the Advancement of Science Annual General Meeting** (AAAS, 1515 Massachusetts Avenue, NW, Washington DC 20005).

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